CHARACTERIZATION OF *DESMODIUM HETEROCARPON* (L) DC. AND *DESMODIUM OVALIFOLIUM* WALL.

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1988

ACKNOWLEDGMENTS

The author thanks Dr. Kenneth Quesenberry for guidance thoughout this program of study and research. Committee members Dr. David Knauft, Dr. Gloria Moore, Dr. Carrol Chambliss, and Dr. Paul Lyrene offered sensible advice for bridging murky matters and avoiding deadends.

Dr. Rex Smith made much of the isozyme work possible by allowing the author generous use of his lab and his knowledge of the subject. Dr. Albert Kretschmer provided insight into the genus Desmodium and triggered new thoughts on the subject with his very specific questions.

The author thanks Caryl McKellar for knowing when patience and support were beneficial, and when lack of patience was better.

David Moon deserves credit for making much of the day-to-day research run smoothly and pleasantly.

Special thanks to Dr. Barbara Probert and the Math Confidence Workshop, without which none of this would have been possible.

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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

CHARACTERIZATION OF DESMODIUM HETEROCARPON (L) DC. AND DESMODIUM OVALIFOLIUM WALL.

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December 1988

Chairman: K. H. Quesenberry
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Characterization of Desmodium heterocarpon (Dh) and D. ovalifolium (Do) germplasm was undertaken as the first step of a breeding program. The objectives were to characterize Dh, Do, and five interspecific hybrids for morphological traits, isozyme markers, cytological behavior during meiosis, pollen viability, and reproductive biology.

Fifty-seven lines of Dh and six lines of Do were grown in two replications in the field in Gainesville, Florida, in 1985, or in two replications in the field in 1986 in Dschang, Cameroon, or in both locations. Plant height, plant width, leaf length, leaf width, flowering date, and presence of marked leaves were measured. Plant height ranged from 9 to 125 cm. Plant width varied from 13 to 78 cm. Leaf length varied from 2.9 to 6.5 cm. Leaf width varied from 1.4 to 3.4 cm. Two lines of D. heterocarpon subspecies angustifolium had leaf length/width ratios > 3.0 (a major taxonomic descriptor delineating this subspecies),

whereas a third line was < 1.5 in Gainesville and Cameroon. Caged Do set little or no seed; caged Dh, and uncaged Do and Dh, produced seed. Pollen stainability for parental and hybrid lines was high.

Two Do lines, three Dh lines, and five Do x Dh hybrids were grown in 10 cm pots both in a shade house and in a greenhouse in 1987-1988. Meiotic analysis showed univalents at metaphase I and laggards at first division segregation in two of the five hybrids. Starch gel electrophoretic analysis of isozyme markers in root and leaf material showed staining differences for peroxidase between parental lines and hybrids. Root material showed differences for phosphohexose isomerase between parental lines and hybrids.

Segregation for marked leaf in a Do x Dh F_2 progeny was 9:7, marked leaf:unmarked leaf. Modal F_2 flowering date was midway between parental flowering dates, but individual plant flowering dates ranged over one month. Segregation for flower color in a Dh x Dh cross was 3:1 purple:white.

These findings suggest that although Do and Dh are separated by morphological differences, crossing barriers between the two species are low. Morphological and isozyme markers identified in this research will aid in recognizing successful hybrids.

CHAPTER I
INTRODUCTION

Tropical forage legumes research in Florida accelerated in the early 1970s as a result of rising prices for crude oil which meant rising costs for nitrogen fertilizer derived from oil and natural gas. This increased cost made research into alternative sources of nitrogen fertilizer more attractive, particularly research into tropical forage legumes that fix nitrogen symbiotically. Choice of a forage legume is complicated by disparate seasonal temperature variation in Florida. Tropical summer temperatures and cold winter temperatures make it unlikely that one species can provide high quality forage throughout the year. However, a perennial tropical forage legume could improve forage crude protein during mid and late summer when traditional forage grasses are of low nutritional content (Sollenberger et al., 1988). The genus Desmodium contains several tropical forage legumes with major agronomic potential: D. canum (Gmel.) Schin. and Thell., D. intortum (Mill.) Urb., D. ovalifolium Wall., D. sandwicense E. Mey., and D. uncinatum (Jacq.) DC. (Imrie et al., 1983).

Desmodium is a large genus containing an undermined number of species variously listed as 200 (Takahashi, 1952), 350 (Bryan, 1966), 350-450 (Imrie et al., 1983), and 500

(Younge et al., 1964). Difficulty enumerating the species number stems from the extreme variation in morphological characteristics within the genus that extends across species delineations (Imrie et al. 1983). Contributing to the problem is the existance of two widely separated major centers of origin; the southeast Pacific and central America. No complete monograph is available on Desmodium. The Asian species have been clasified extensively by Ohashi (1973) and the American species have been studied primarily by Shubert (1963).

Desmodium heterocarpon, which has received some research attention in Florida, is a case where questions remain as to the exact delineation of species. Ohashi (1973) described two subspecies of D. heterocarpon, one with four varieties. The variety D. heterocarpon subspecies heterocarpon variety heterocarpon includes both lines typified by 'Florida" carpon desmodium [D. heterocarpon (L.) DC. variety heterocarpon] as well as the species described as D. ovalifolium Wall. The distinguishing characteristics in Ohashi's key that grouped these two species together in one subspecies were pods distinctly hooked-hairy, inflorescence-rachides covered with spreading hooked hairs, inflorescences densely flowered and pedicles 2-5(-8) mm long in fruit. This classification reduced D. ovalifolium Wall. to variety status, although it was listed as a separate species by Schindler in 1928 (Ohashi, 1973) and contnues to be referred to as a separate species. Simultaneously, a

second D. ovalifolium, this one with authority Guill and Perr., has been referenced (Hussain et al., 1987). Schubert (1971) listed D. ovalifolium Guill and Perr. (described in 1832) as synomymous with D. adscendens (Sw.) DC. described in 1825. Characteristics of Shubert;s D. adscendens were herbaceous growth, creeping or forming tussocks, and leaves that are pilose to glabrous on top and densely pilose on the bottom. Shubert's description of D. heterocarpon was shrubby or with procumbent habit with leaves glabrous to moderately pilose above and sparsely to abundantly pilose below. No mention was made of marked leaves in either species by Shubert or Ohashi. Ohashi (1973) did not list D. ovalifolium Guill and Perr. among the D. ovalifolium synonymous with D. heterocarpon ssp. heterocarpon var. heterocarpon, therefore the exact relationship between the D. ovalifolium of Wall. and Guill and Perr. remains unknown.

The major distinctions between the two species are at the gross morphological level. Imrie used D. ovalifolium in his discussion of the genus because the agronomic type is known by that name in Southeast Asia (Imrie et al., 1983). Characteristics that distinguish D. ovalifolium from D. heterocarpon are reported as prostrate versus erect growth habit; leaves unifoliate when young versus trifoliate when young, leaves glabrous on top versus opaque on top, leaves unmarked versus marked with light green or white markings; inflorescenses compact versus elongated; pods densely pubescent versus glabrous to slightly pubescent

(Schultze-Kraft and Benavides, personal communication). These wide morphological differences between D. ovalifolium and D. heterocarpon have resulted in most researchers continuing to refer to the agronomic types as distince species (Schultze-Kraft and Benavides, personal communication). In this dissertation the specific rank of D. ovalifolium will be maintained.

The genus has an even distribution across latitudes with 160 species in equatorial regions and 110 species in temperate regions according to Index Kewensis (Imrie et al., 1983). The center of origin of D. heterocarpon is reported as Asia and the Pacific Islands (Kretschmer et al., 1979) although it is now found in Africa, Australia, the Pacific Islands, and Asia (Imrie et al., 1983). Recent collections of D. ovalifolium have been made at its presumed center of origin in Thailand and Malaysia (Schultze-Kraft and Pattanavibul, 1985).

Desmodium heterocarpon originated in tropical latitudes although high altitudes within these latitudes may expose plants to cool weather and light frosts. Desmodium heterocarpon has survived frosts and air temperatures down to -6.6^{o}C (Kretschmer et al., 1979). Similar climate in Florida and Australia at equivalent latitudes suggests that tropical legume data from Australia should be applicable to Florida (Kretschmer, 1970). Based on this, research was started on D. sandwicense and D. heterocarpon at Fort Pierce, Florida.

Kretschmer (1970) found D. intortum to be higher yielding in asociation with Pangola grass (13,750 kg/ha) than D. heterocarpon which produced 9,990 kg/ha. Desmodium heterocarpon was compared again with D. intortum 'Greenleaf' in association with three grasses and did not produce as well as D. intortum (Kretschmer et al., 1973; Kretschmer et al., 1976). Desmodium hetercarpon grown with 'Pangola' digitgrass (Digitaria decumbens Stent.) or 'Pensacola' bahiagrass (Paspalum notatum Flugge.) had total annual dry matter yields of 9680 abd 9020 kg ha^{-1}, respectively (Kretschmer et al., 1979). A comparison of seven tropical legumes with mixtures of six tropical grasses demonstrated that D. heterocarpon outyielded many of the other legumes in the summer, but when seed formation began in the fall, yield often dropped below that of the other legumes tested (Kretshmer et al., 1982).

Desmodium ovalifolium is reported to have drought tolerance, shade tolerance, adaption to acid soils, and persistance under grazing (Imrie et al., 1983). It has produced over 7,000 kg/ha in eight months in Colombia (Imrie et al., 1983). Contributing to high yield and persistance under grazing is plant morphology. Bryan (1966) reported that the large, axillary buds in D. uncinatum and D. intortum provided rapid regrowth when the stem tip and leaves were removed under light grazing but that removal of much of the stem as happens under heavy grazing greatly retards regrowth of both species. Therefore difference in

stocking intensity may influence legume persistence. Kretschmer (1979) reported that D. heterocarpon persisted in fields planted with 'Coastcross-1' bermudagrass (Cynodon dactylon (L.) Pers.) for three reasons: the cattle preferred 'Coastcross-1' to D. heterocarpon, the growth form of D. heterocarpon was prostrate under grazing, and the crowns from which the plant regrew were situated low to the ground.

Crude protein in Desmodium heterocarpon was significantly lower than in D. intortum or 'Siratro' [Macroptilium atropurpureum (DC) URB] when separately grown with each of three torpical grasses (Kretschmer et al., 1973). Kretschmer et al. (1976) looked at seven legumes in association with five grasses and determined that D. heterocarpon produced the lowest amount of crude protein and had the lowest in vitro organic matter digestion (IVOMD) of the seven legumes tested, although all combinations were above the seven percent crude protein suggested by Minson adn Milford (1967) as necessary to sustain normal intake of tropcial grasses. In the same experiment feeding pellets of D. heterocarpon to cattle resulted in low digestibility, which could possibly have been an artifact of the handling and processing. The IVOMD ranged from 46.7 to 48.2 percent.

Toxicity is another aspect of Desmodium forage quality that has received attention. Reports on Desmodium species indicate toxicity levels are low. Zoebischh et al. (1952) fed 25 species of Desmodium (species unspecified) to chicks and reported that none of the 25 species reduced

chick growth, although some intoxication was evident with eight of the species. Bryan (1966) reported low alkaloid determinations on D. uncinatum, small amounts of oestrogens in D. uncinatum and D. intortum, and no reports of adverse effects form feeding Desmodium in Australia. Tannin levels in D. uncinatum and D. intortum border on levels that according to Rotar are high enough to interfere with rumen cellulase activity but according to Hutton and Coote are not high enough to interfere with rumen cellulase activity (Bryan, 1969). Recent work with D. heterocarpon and D. ovalifolium indicated that tannin levels varied by 100% in 42 lines of D. heterocarpon tested, and by 50% in 20 lines of D. ovalifolium lines tested (Albrecht and Quesenberry, 1987).

Researchers have also looked at factors that may reduce yield and stand persistence such as pest and disease susceptibility. Some causes of lack of persistence in Desmodium in general are soil moisture stress during the growing season, insect attack, poor seedling vigor, frost, and competition from grasses (Imrie et al., 1983).

Web-worms and nematodes are the major pest problem of D. heterocarpon in Florida. Desmodium heterocarpon suffers from web-worm attacks on leaves and flowers, especially if large amounts of leaf material accumulate (Kretschmer et al., 1976).

Root-knot nematodes, Meloidogyne sp., are prevalent in Florida, particularly in areas that have been used for

vegetable production before conversion to pastures. Resistance to root-knot nematode varies among and within Desmodium species. Desmodium heterocarpon showed mixed resistance to root-knot nematodes in Florida (Kretschmer et al., 1980). This test of seven accessions of D. heterocarpon showed that some lines were tolerant to root-knot nematode whereas 'Florida' is highy susceptible to root-knot nematode. The nematode tolerant D. heterocarpon accessions yielded less than 'Florida' when all were grown without nematodes, but their yield was equal to or higher than 'Florida' when grown in the presence of root-knot nematodes. Desmodium intortum in this test showed resistant to root-knot neamtode. Baltensperger et al. (1983) reported that one line of D. uncinatum was highly resistance to M. incognita, M. arenaria, and M. javanica. In the same experiment, four D. heterocarpon lines [including 'Florida' and a line identified by Kretschmer et al. (1980) as susceptible] were highy susceptible to all three root-knot nematodes, D. ovalifolium (U.F. 28) was slightly resistant to M. incognita, and D. ovalifolium IRFL 1699 had some resistance to all three lines. Desmodium uncinatum 'Silverleaf' was susceptible to M. javanica and M. hapla (no authority given), highly susceptible to M. arenaria, and resistant to M. incognita incognita and M. incognita acrita (no authority given) (Minton et al., 1967). Tests in Colombia indicated that D. heterocarpon and D. ovalifolium were susceptible to Meloidogyne javanica (Treub) Chitwood

(Lenne, 1981). Recent testing of D. heterocarpon and D. ovalifolium response to Meloidogyne arenaria race 1 (Neal) Chitwood, Meloidogyne incognita race 3 (Kofoid and White) Chitwood and M. javanica showed that both types varied among lines for number of galls and number of egg masses (Queseberry and Dunn, 1987). In this experiment, the highest level of resistance in D. heterocarpon and D. ovalifolium to all three root-knot species was identified in D. ovalifolium.

Successful nitrogen fixation by legumes depends on an association with the appropriate Rhizobium spp. Introduction of D. heterocarpon into Florida must proceed with the knowledge that a compatible strain of Rhizobium spp. already exists in the soil or can be added with the D. heterocarpon. The situation regarding Rhizobium spp. nodulation of Desmodium in general is somewhat ambiguous. Both D. uncinatum and D. intortum are reported to have rhizobium requirements that are usually met by native soil populations (Imrie et al., 1983). However in some cases results were poor when D. intortum was planted without inoculation. In other cases inoculation improved nodulation but not yield. Additional work has shown that inoculation with a specific rhizobium will generate only 5% of the total nodules if a compatible native strain is present, but will generate 100% of the nodules if no native compatible strain is present. Kretschmer (1970) suggested that Florida soils generally have sufficient native and introduced legumes for

which, except for virgin soils, inoculation should not be necessary. Where necessary, a "cowpea" type should be used at twice the recommended rate.

Desmodium heterocarpon is slow to establish even under ideal conditions (Kretschmer et al., 1979; Kretschmer et al., 1982). Poor establishment of D. heterocarpon was also encountered in experiments with legume-bahia grass pastures at Ona, Florida (Pitman, 1983). Although the plants produced a good seed set and survived well under grazing, the initial difficulty with establishment has given D. heterocarpon mixed acceptance among growers in Florida (Pitman, 1986).

Traits of interest in a genetic improvement program in D. heterocarpon are flowering time, chromosome number, reproductive compatibility between types and species, and inheritance of important traits. The flowering time for D. heterocarpon appears to be determined by photoperiod. Desmodium heterocarpon is triggered to flower by shortening days. The chromosome number of all Desmodium species is 2n=22 (Rotar and Urata, 1967). Schifino (1983) investigated two morphological forms of D. uncinatum that had the same 2n number. The presence of similar numbers of chromosomes in species and lines suggests the possibility of interspecific crosses between Desmodium species. Chromosome pairing failure has been reported in crosses between D. intortum and D. sandwicense (Hacker, 1968). No evidence of self-incompatibility has been reported (Imrie et al., 1983).

Most Desmodium spp. are thought to be primarily self-pollinated and that, along with the minute size of the flowers, makes artificial hybridization difficult. Cytoplasmic male sterility has been reported in D. sandwicense with restorer genes in D. intortum (McWhirter, 1969).

Flower color, leaf markings, and stem color are controlled by a single gene pair whereas seed size, internode length, raceme length, and nodulating ability are thought to be polygenically controlled in D. intortum and D. uncinatum (Rotar and Chow, 1971).

Interspecific crosses are often made to introduce desirable genes from one species into another. In Desmodium, crossing occurred naturally between D. intortum and D. sandwicense. Desmodium intortum 'Greenleaf' is a combination of three lines, one of which is now known to have contained some genes introgressed from D. sandwicense. The first indication of the presence of these genes was a shift in date of flowering and yield in the population as these introgressed genes were selected against (Imrie and Blogg, 1982).

Desmodium heterocarpon offers promise for growers in Florida, more so if problems involving establishment and nematode susceptibility can be worked out. The objectives of this research were to characterize D. heterocarpon and D. ovalifolium as the basis for initiating a breeding program to combine desired traits of both species. Specific

objectives were: (1)to determine range in morphological variation by quantifying leaf size, plant size, flowering date, stem color, and presence of marked leaves; (2)to improve the ability to make crosses by determining percentage outcrossing, pollen viability, and chromosome behavior during meiosis; and (3)to develop a method for verifying hybrids by using isozyme markers.

CHAPTER II
MORPHOLOGY OF *DESMODIUM HETEROCARPON* AND *DESMODIUM OVALIFOLIUM*

The International Board for Plant Genetic Resources (IBPGR) has published a list of descriptors for forage legumes (Andersen and Davies 1984). These descriptors are divided into four categories for accession data, collection data, characterization data, and preliminary evaluation data. These descriptors will, in part, alert researchers involved with germplasm to the range of information needed about each accession, as well as standardize the responses so that results can be universally understood. Some examples are given for white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), alsike clover (*Trifolium hybridum* L.), lucerne (*Medicago sativa* L.), and birdsfoot-trefoil (*Lotus corniculatus* L.). Although these descriptors were not written with *Desmodium* in mind, the characterization descriptors indicate aspects of forage legumes that are thought to be important. These include sowing and planting date; evaluation planting design used; length, width, and shape of leaf; presence of marked leaves; growth habit; and date and color of flowers.

Current collections of *Desmodium* include over 11,000 samples in Australia and over 12,000 in Colombia, followed by 830 in Brazil and 230 in the U.S.A. (Davies, 1984).

Desmodium uncinatum (Jacq.) DC., D. intortum (Mill.) Urb., and D. ovalifolium Wall. are among legumes listed as first priority for further collection and evaluation in tropical and subtropical areas of South America, Mexico and Central America, Asia, and Australia (Davies, 1984). Attempts to collect D. ovalifolium and D. heterocarpon (L) DC. germplasm from Southeast Asia have resulted from cooperative efforts by several research organizations including the Thailand Institute of Scientific and Technological Research, Livestock Department (Bangkok), the Centro Internacional de Agriculture Tropical (CIAT), the Malaysian Agricultural Research and Development Institute, and IBPGR. Most recently, Schultze-Kraft and Pattanavibul (1985) collected six samples of D. heterocarpon and 12 samples of D. ovalifolium in eastern Thailand (Schultze-Kraft and Pattanavibul, 1985). Desmodium germplasm was evaluated at CIAT by Sobrinho (1982). He evaluated 18 accessions of D. ovalifolium and one accession of D. heterocarpon and found highly significant differences among accessions for all parameters analyzed including dry matter yield and crude protein. Sobrinho also reported in this study that the one D. heterocarpon accession appeared morphologically and biochemically different from the 18 D. ovalifolium accessions studied. Schultze-Kraft (personal communication) evaluated 83 accessions of D. ovalifolium for characters such as establishment rate, date of flowering, dry matter yields, plant height and lateral growth, and chemical

analyses. Schultze-Kraft noted continuous morphological variation between D. ovalifolium and D. heterocarpon but attributed this to natural hybrids between the two species.

Hybridization between Desmodium species has been reported by several researchers (Chow and Crowder, 1972; Chow and Crowder, 1973; Hutton and Gray, 1967; Park and Rotar, 1968; Rotar and Chow, 1971). The marked leaf in D. heterocarpon is a possible morphological marker for crosses with plants with unmarked leaves. Silver leaf-mark of D. uncinatum was dominant in crosses with D. intortum and D. sandwicense (Chow and Crowder, 1973). Brown fleck leaf-mark of D. intortum was dominant in crosses with D. sandwicense E. Mey. and D. uncinatum (Chow and Crowder, 1973). Hutton and Gray (1967) reported similar 3:1 segregation ratios for silver and brown fleck leaf-marks in F_2 generations originating from D. uncinatum and D. intortum crosses. Park and Rotar (1968) divided flower color in D. sandwicense into a white group and a purple group. F_2 segregation ratios were 3:1 for purple over white.

The objectives of this research were threefold: (1)to characterize the D. heterocarpon and D. ovalifolium germplasm collection at the University of Florida, Gainesville, for the morphological characters leaf width, leaf length, leaf length/width ratio, plant height, plant width, presence of marked leaves, and for reproductive biology; (2)to determine segregation ratios for marked leaf and flower color in the F_2 population; and (3)to determine date of flowering in an F_2 population.

Materials and Methods

Three field experiments were conducted to morphologically describe fifty-seven lines of D. heterocarpon and six lines of D. ovalifolium. Seeds of D. heterocarpon and D. ovalifolium lines were obtained from Dr. A. E. Kretschmer, Jr., Agricultural Research Center, Ft. Pierce, Florida; from the USDA Plant Introduction Service, Experiment, Georgia; and from CIAT, Colombia. All line identification numbers will be IRFL numbers unless specified otherwise. Table 2.1. cross-references U.F., IRFL, and CIAT identification numbers of material evaluated and location where evaluated. F_1 seed of crosses 191 (D. ovalifolium U.F. 28 x D. heterocarpon 6105) and 18/185 (D. heterocarpon purple flowered 6106 x D. heterocarpon white flowered 6150) and F_2 seed of 191 and 18/185 were produced by Dr. K. Quesenberry, University of Florida, Gainesville, Florida. Seeds were scarified and then germinated in petri dishes. Individual plants of some lines were isolated in 0.5 x 0.5 x 0.5 m wood frame cages covered with window screening.

Experiment 1. Plants from seed germinated on May 5, 1985, in Gainesville, Florida, were moved to the greenhouse one week later. Five seedlings per row of each accession were planted in the field on June 6, 1985, in a randomized complete block design of two replications. The plants were spaced 0.5 meters in the row and 1.0 meters between rows.

Table 2.1. *Desmodium heterocarpon* and *D. ovalifolium* introduction numbers and location where evaluated.

Introduction Numbers[a]				
UF	IRFL	CIAT	Species[b]	Location[b]
20	588,6102	365	H	C,F
137	854,6104	3653	H/h/h	F
143	1695,6105	3669	H	F
144	1696,6106	3670	H/h/str	C,F
145	1697,6117	3671	H/h/h	F
146	1699	3673	O	F
28	1946	3674	O	C,F
-	3279		H	C
-	3280		H	C
-	3321	3781	O	C
-	3322	3784	O	C
-	3324	3787	H	C
-	3325	3788	O	C
-	3661	3755	H/h/str	C
-	3663	3785	H	C
-	3664	3793	O	C
-	3776	-	H	C
136	6103	3116	H	F
449	6107	3672	H/h/h	F
-	6108	3675	H/h/h	F
444	6109	3678	H/h/str	C,F
445	6110	3680	H	F
-	6111	3687	H	F
447	6112	3688	H	F
-	6113	3700	H/h/h	F
-	6114	3735	H	F
-	6115	3749	H/h/h	F
-	6118	3786	H	F
-	6119	3810	H	C,F
-	6121	3843	H	C,F
-	6122	3984	H	C,F
-	6123	13142	H/a	C,F
-	6124	13146	H	C,F
-	6125	13148	H/a	C,F
-	6135	13162	H/h/h	C,F
-	6141	13170	H	C,F
-	6143	13173	H/a	C,F
-	6144	13175	H/h/str	C,F
-	6146	13177	H	C,F
-	6147	13179	H/h/str	C,F
-	6148	13180	H/h/str	C,F
-	6149	13182	H/h/str	C

Table 2.1. Continued.

Introduction Numbers				
UF	IRFL	CIAT	Species	Location
-	6150	13183	H/h/h	C,F
-	6151	13184	H/h/h	C,F
-	6152	13185	H/h/h	C,F
-	6153	13186	H/h/str	C,F
-	6154	13187	H/h/str	C
-	6155	13189	H/h/str	C,F
-	6158	13119	H	C,F

[a] Plant introduction numbers are numbers assigned by Dr. K. H. Quesenberry, Agronomy Department, University of Florida (UF), by Dr. A. E. Kretschmer, Jr., AREC, Ft. Pierce, Florida (IRFL), and by the Tropical Pastures Program, Columbia (CIAT).

[b] Species: H = species heterocarpon
O = species ovalifolium
H/a = species heterocarpon subspecies angustifolium
H/h/h = species heterocarpon subspecies heterocarpon variety heterocarpon
H/h/str = species heterocarpon subspecies heterocarpon variety strigosum

[c] Location evaluated: C = Cameroon, F = Florida.

The soil type was a Wachula sand (sandy, siliceous, hyperthermic Ultic Haplaquad). Notes were taken on plant height, plant width, leaf length, leaf width, flowering date, and presence of marked leaf on August 15, 1985. Leaf length to leaf width ratio was calculated for each leaf measured. Flowering was noted on dates August 15, September 4, September 12, September 19, October 3, October 23. Individual plants of line 6155 D. heterocarpon, line CIAT 13110 D. ovalifolium, and line CIAT 3670 D. heterocarpon were caged from October 22, 1986, until November 5, 1986. Fertilization was evaluated in terms of number of pods formed, and presence of seeds in the pods.

Experiment II. Seed was germinated in Dschang, Cameroon on Dec. 3, 1986, and transplanted to peat pots one week later. Seedlings in peat pots were moved outside each morning and returned to the lab each evening. Five seedlings of each accession were planted per row in the field on Jan. 20, 1986 in a completely randomized design with two replications. The plants were spaced 0.5 meter apart in the row and 1.0 meter between rows. Notes were taken on plant height, plant width, leaf length, leaf width, and marked leaf, on May 21. Flowering date was recorded noted on May 21, July 21, October 23, 1986, and January 10, 1987. Individual plants of lines 3325 D. ovalifolium and 1699 D. ovalifolium were caged from July 5, 1986 until August 4, 1986. Successful pollination was evaluated in terms of number of pods formed, number of segments per pod,

and presence of seed.

Experiment III: Seed was germinated on May 15, 1987, in Gainesville, Florida. Seedlings were moved to the greenhouse, then to the field on June 20, 1987. Forty-nine F_2 plants of hybrid 191, 45 F_2 plants of hybrid 18/185, 7 ramets of F_1 hybrid 191, 9 plants of IRFL 6105, and 9 plants of 6102 were planted 0.5 m apart in the row and 1.0 m between rows. Data on segregation for marked leaf among 191 F_2's were taken on September 3, 1987. Data on flowering date of $F_2$191 were taken on September 13 and 19, October 3, 15, 20, and 28, and November 3 and 17, 1987. Data on flower color of 18/185 F_1 were taken as plants came into flower, either in the field, or later, in the greenhouse. Three plants of 6105 D. heterocarpon and three plants of 6102 D. heterocarpon were caged on September 1, 1987. Seed weight produced per plant was used to evaluate mode of pollination.

In addition to caging data, notes were taken on greenhouse seed set for lines listed in Table 2.2. Accessions in flower in the greenhouse between January 18, 1988, and September 20, 1988, were periodically observed for percentage successful pod formation. Pod formation was noted four days after flower opening. Lines 6150, U.F. 28, U.F. 160, and hybrids 195, 191 which did not self-trip early in the morning, were tripped and self-pollinated. Lines 6150. U.F. 28, U.F. 160, and hybrids 195, 191, 83-4-3, 83-4-2 were tripped and cross-pollinated with another plant

Table 2.2. Pod set on Desmodium in the greenhouse with unmanipulated, self pollinated, or cross pollinated flowers.

	Unmanipulated			Self Pollinated			Cross pollinated		
line	flowers	pods	pod set	flowers	pods	pod set	flowers	pods	pod set
	-----no.-----		--%--	-----no.-----		--%--	-----no.-----		--%--
6102	81	6	07						
6105	56	14	25						
6150	124	0	0	60	7	11	25	2	08
U.F. 28	127	0	0	30	22	73	64	37	57
U. F. 160	214	0	0	110	67	60	37	16	43
F_1 Hybrids									
83-4-3	65	0	0				40	8	20
83-4-2	188	1	0				56	14	25
195	25	1	04	271	37	17	105	17	16
191	77	1	01	880	216	24	52	9	17

of the same line. Successful pod formation was noted four days later.

Results

Plant height, plant width, leaf length, leaf width, leaf length/width ratio, flower date, and presence of marked leaf for D. heterocarpon and D. ovalifolium grown in Gainesville in 1985 are listed in Table 2.3. Although plant height ranged from 9 cm to 39 cm when these measurements were taken, some lines eventually grew to mean heights of 1 meter before frost. Plant width ranged from 13 cm to 84 cm. Leaf length varied from 2.8 to 6.5 cm. Leaf width ranged from 2.7 to 6.7 cm. Three lines (6123, 6124, 6125) had leaf length/width ratios of higher than 3.0 cm. Two lines (6123, 6125) were D. heterocarpon ssp. angustifolium. The third line was specified as D. heterocarpon without subspecies or variety ranking. A third line (6143) identified by CIAT as D. heterocarpon ssp. angustifolium had a leaf length/width ratio of only 1.4. Leaf length/width averages ranged from 1.0 to 3.7.

The majority of D. heterocarpon lines screened had marked leaves. Most D. ovalifolium and D. heterocarpon ssp. angustifolium had unmarked leaves. One line of D. heterocarpon spp. heterocarpon var. heterocarpon (6108) and one line of D. heterocarpon species and variety unspecified (6158) had unmarked leaves.

Table 2.3. *Desmodium heterocarpon* and *D. ovalifolium* morphological measurements taken 102 days after planting in Gainesville, Florida, 1985.

Line	Plant Height	Plant Width	Leaf Length	Leaf Width	Leaf Ratio	Marked Leaf[a]	Flowering Date[b]	Type[c]
	- - - - - - cm - - - - - -							
UF 28	17	54	4.0	4.7	1.7	1	-	O
UF 145	17	63	4.7	6.0	1.6	2	8/15	H/h/h
UF 146	9	31	3.5	4.7	1.5	1	-	O
UF 449	24	60	5.0	5.5	0.9	2	9/19	H/h/h
6102	22	64	4.8	6.0	1.6	2	9/12	H
6103	20	62	4.3	4.7	1.9	2	9/12	H
6104	21	84	4.2	5.0	0.8	2	9/4	H/h/h
6105	16	51	4.0	5.2	1.5	2	9/19	H
6106	24	26	4.0	3.7	2.1	1	10/3	H/h/str
6107	27	78	4.7	5.5	1.7	2	9/4	H/h/h
6108	17	29	3.1	3.7	1.7	1	9/4	H/h/h
6109	21	59	4.5	5.0	1.8	2	9/4	H/h/h
6110	16	41	3.8	4.7	1.6	2	9/4	H
6111	21	67	4.5	5.2	1.7	2	9/19	H
6112	17	70	5.3	6.2	1.7	2	9/12	H
6113	10	25	2.8	4.0	1.4	2	9/12	H/h/h
6114	15	38	3.6	4.5	1.6	2	9/19	H
6115	18	37	3.1	6.7	1.0	2	9/4	H/h/h
6117	22	36	4.2	5.0	1.7	2	10/3	H/h/h
6118	39	39	4.5	4.5	2.0	2	10/23	H
6119	24	35	4.1	3.5	2.4	2	10/23	H
6121	30	21	4.0	4.0	2.0	2	10/23	H
6122	16	30	3.2	3.5	1.9	2	9/4	H
6123	24	17	5.5	3.2	3.4	1	10/23	H/a

Table 2.3. continued

Line	Plant Height	Plant Width	Leaf Length	Leaf Width	Leaf Ratio	Marked Leaf	Flowering Date	Type
	- - - - - - - - cm - - - - - - - -							
6124	23	21	6.5	3.5	3.7	2	10/23	H
6125	20	13	4.8	2.7	3.5	1	10/23	H/a
6135	19	17	4.0	3.7	2.1	2	10/23	H/h/h
6141	36	40	4.1	3.7	2.2	2	10/23	H
6143	24	28	5.7	4.0	1.4	1	10/23	H/a
6144	33	38	5.2	5.5	1.9	2	11/14	H/h/str
6146	27	17	4.8	4.7	2.0	2	11/14	H
6147	22	32	3.8	3.5	2.2	2	10/23	H/h/str
6148	15	27	4.8	4.2	2.3	2	11/14	H/h/str
6150	31	22	3.6	3.0	2.4	2	10/23	H/h/h
6151	24	27	4.0	3.2	2.5	2	10/23	H/h/h
6152	24	38	4.1	4.0	2.0	2	11/14	H/h/h
6155	27	55	4.6	4.2	2.2	2	10/23	H/h/str
6149	25	34	5.7	4.7	2.4	2	11/14	H/h/str
6153	16	41	4.5	4.2	2.1	2	11/14	H/h/str
6158	13	30	3.2	3.7	1.7	1	10/23	H
LSD (P=0.05)	8.8	19	1.0	0.7	0.4			

[a] Leafmark: 1= unmarked leaf.
2= marked leaf (lightgreen to white, irregular shaped marks)

[b] Not all lines flowered.

[c] Type: H = species heterocarpon
O = species ovalifolium
H/a = species heterocarpon subspecies angustifolium
H/h/str = species heterocarpon subspecies heterocarpon variety strigosum.
H/h/h = speceis hetercarpon subspecies heterocarpon variety heterocarpon.

Flowering started with line U.F. 145 on August 15. Neither D. ovalifolium line (U.F.28 and U.F.146) flowered before November 4, 1985. Other D. ovalifolium lines observed in other years have shown flowering dates of Nov. 1 to Nov. 30. Ten lines flowered between mid-September and mid-October. Twenty lines started flowering between mid-October and mid-November.

Results of pod set as influenced by caging are given in Table 2.4. Three plants each of D. heterocarpon ssp. heterocarpon var. strigosum 6106 and 6155 were enclosed in individual screened cages to exclude insect pollinators while five plants of each line were left uncaged. Pod set for 6155 was high for both caged and uncaged plants. Pod set for caged (3,646) and uncaged (4,189) was not different ($P>0.05$). Pod set for 6106 for caged (28) and uncaged (1,142) was different ($P<0.05$). Three plants of six D. ovalifolium (CIAT 13110) enclosed in individual screen cages produced a mean of 34 legumes. Three uncaged plants produced a mean of 1705 legumes per plant that was different at $P=0.10$.

Plant height, plant width, leaf length, leaf width, presence of marked leaves, leaf length/width average, and flower date for D. ovalifolium and D. heterocarpon grown in Cameroon in 1986 are shown in Table 2.5. Plant height varied from 8 to 125 cm. Plant width varied from 52 to 240 cm. Leaf length ranged from 2.6 to 6.4 cm. Leaf width varied from 1.2 to 3.4 cm. Leaf length/width average varied

Table 2.4. *Desmodium heterocarpon* and *D. ovalifolium* caged and uncaged pod set in Gainesville in 1986.

Line Number	Plant	Caged	No. of Pods	Seed present
IRFL 6155	1	yes	1,056	yes
D. heterocarpon	2	yes	912	yes
	3	yes	1,678	yes
	4	no	700	yes
	5	no	1,443	yes
	6	no	279	yes
	7	no	2,204	yes
	8	no	293	yes
CIAT 13110	1	yes	46	yes
D. ovalifolium	2	yes	22	yes
	3	yes	33	yes
	4	no	117	yes
	5	no	2,000+	yes
	6	no	3,001	yes
IRFL 6106	1	yes	8	yes
D. heterocarpon	2	yes	2	no
	3	yes	18	yes
	4	no	23	yes
	5	no	59	yes
	6	no	16	yes
	7	no	751	yes
	8	no	293	yes

[a] mean number of legumes between caged and uncaged was not different ($P>0.05$) for 6155, was different ($P=0.10$) for 13110, and was different ($P<0.05$) for 6106.

Table 2.5. *Desmodium heterocarpon* and *D. ovalifolium* morphological measurements taken 170 days after planting in Dschang, Cameroon, 1986.

Line	Plant Height	Plant Width	Leaf Length	Leaf Width	Leaf Ratio	Marked Leaf[a]	Flowering Date[b]	Type[c]
	- - - - - -cm- - - - - - -							
U.F.28	13	110	5.5	3.2	1.5	1	-	O
U.F.146	12	85	3.8	2.6	1.5	2	5/21	O
U.F.449	27	145	3.8	2.2	1.7	1	-	H
6102	42	180	4.5	2.5	1.8	2	10/23	H
6103	23	140	3.4	2.0	1.7	2	-	H
6106	21	113	4.0	2.0	2.0	1	-	H
6109	34	77	4.6	2.4	1.9	2		H
3279	25	130	4.2	2.8	1.5	1	7/21	H
3280	15	84	4.0	2.4	1.7	1	-	H
3321	12	170	4.0	2.4	1.7	1	-	O
3322	15	155	3.7	2.3	1.6	1	7/21	O
3324	61	125	4.0	1.6	2.5	2	-	H
3325	10	170	4.9	2.6	1.9	1	5/21	O
3661	45	52	4.1	2.2	1.9	2	7/21	H/h/str
3663	106	145	4.4	2.1	2.0	2	10/23	H
3664	77	210	4.0	1.7	2.4	2	-	O
3776	18	155	3.2	2.0	1.6	2	-	H
6119	85	101	4.1	1.9	2.2	2	-	H
6121	95	160	4.2	1.8	2.3	2	-	H
6122	17	80	2.6	1.4	1.8	2	-	H
6123	87	65	6.4	1.4	4.6	1	-	H/a
6124	87	72	7.0	1.6	4.4	2	10/23	H

Table 2.5. continued

Line	Plant Height	Plant Width	Leaf Length	Leaf Width	Leaf Ratio	Marked Leaf	Flowering Date	Type
	- - - - - - - cm - - - - - - -							
6125	45	100	5.0	1.2	4.1	1		H/a
6135	87	117	4.8	2.1	2.3	2	-	H
6141	112	187	4.4	1.8	2.4	2	10/23	H
6143	52	55	2.8	3.4	0.8	1	-	H/a
6144	107	96	3.6	2.8	1.3	2	10/23	H/h/str
6146	55	127	4.1	1.7	2.4	2	12/10	H
6147	77	175	3.8	1.5	2.5	2	7/21	H/h/str
6148	72	157	4.8	2.1	2.2	2	-	H/h/str
6149	125	175	5.3	2.0	2.6	2	-	H/h/str
6150	65	240	3.3	1.3	2.5	2	7/21	H/h/h
6151	96	155	4.2	1.6	2.6	2	10/23	H/h/h
6152	75	167	4.8	1.9	2.5	2	12/10	H
6153	56	135	4.3	1.8	2.4	2	-	H/h/str
6154	52	155	6.2	2.3	2.7	2	-	H/h/str
6155	62	135	4.2	1.8	2.3	2	-	H/h/str
6158	8	190	4.2	2.5	1.6	1	7/21	H
LSD (P = 0.05)	48	83	1.1	.54	.30			

[a] Leafmark: 1= unmarked leaf
2= marked leaf (lightgreen to white, irregular shaped marks).

[b] Some lines did not flower.

[c] Type: H = species heterocarpon.
O = species ovalifoliumh.
H/a = species heterocarpon subspecies angustifolium.
H/h/str = species heterocarpon subspecies heterocarpon variety strigosum.
H/h/h = species heterocarpon subspecies heterocarpon variety heterocarpon.

from 0.8 to 4.6. Desmodium heterocarpon ssp. heterocaron var. angustifolium lines 6123 and 6125 had leaf length/width averages over 4.0 but 6143 had a leaf length/width average of less than 1.5. All three D. heterocarpon ssp. heterocarpon var. angustifolium accessions had unmarked leaves. Four D. ovalifolium (U. F. 28, 3321, 3322, and 3325) had unmarked leaves but two other lines (U. F. 146 and 3664) had some marked leaves. Sixteen of 37 lines flowered during a year of observation in Cameroon. First flowering occurred on July 21 (D. ovalifolium lines U. F. 146 and 3325) and last flower date was D. heterocarpon 6146 blooming on December 10, 1986.

Three of six plants of D. ovalifolium 3325 and U. F. 146 were enclosed in individual screened cages (Table 2.6.), while three of each line were left uncaged. Caged plants of both lines produced no pods, except for plant # 1 of line 3325. This one pod consisted of one segment that did not contain a seed. The remaining four 3325 and three 146 plants were uncaged, and set pods with seeds, except for the seventh plant of line 3325 and one of the uncaged U. F. 146 plants which were not flowering.

Marked leaf and flower color F_2 segregation data are given in Table 2.7. The observed segregation ratio for presence or absence of marked leaves was approximately 9:7 in the F_2 generation of 191 (U. F. 28 x 6105). The F_1 had marked leaves. The observed segregation ratio for flower color was 3:1 for presence of color versus absence of

Table 2.6. *Desmodium ovalifolium* caged and uncaged pod set in Dschang, Cameroon, in 1986.

Line Number	Plant Number	Caged	Flowering	No. of Pods	Seed Present
IRFL # 3325	1	yes	yes	1	no
D. ovalifolium	2	yes	yes	0	-
	3	yes	yes	0	-
	4	no	yes	42	yes
	5	no	yes	78	yes
	6	no	yes	21	yes
	7	no	no	0	-
U. F. 146	1	yes	yes	0	-
D. ovalifolium	2	yes	yes	0	-
	3	yes	yes	0	-
	4	no	no	0	-
	5	no	yes	43	yes
	6	no	yes	97	yes

Table 2.7. Segregation data for marked leaves in 191 F_2 and flower color 18/185 F_2 in Gainesville, Florida,in 1987.

Hybrid ID[a]	Trait	No. with trait	No. without trait	total	exp. ratio	observed ratio	x^2	P value
191 F_2	Leafmark	23	24	47	9:7	1:1	1.00	P>.25
18/185 F_2	Flower Color	24	8	32	3:1	3:1	0	

[a] 191 is a cross of U.F. 28 D. ovalifolium (unmarked leaves) x 6105 D. heterocarpon (marked leaves)

18/185 is a cross of 6106 D. heterocarpon (purple flowered) x 6150 D. heterocarpon (white flowered)

color in the F_2 generation of hybrid 18/185 (6106 purple flowered x 6150 white flowered).

Frequency distributions for flower dates for 6105, 191 F_1, and 191 F_2 are shown in Table 2.8. Female parent, U. F. 28, was not planted in 1986 in Gainesville. This line, however, did not flower by day 307 in 1985 in Gainesville. 6105 mean flower date for nine plants was day 267. Three ramets of 191 F_1 had a mean flower date of 298 days. Flowering dates for 33 191 F_2 plants had a mean of 293 days, excluding 7 plants that had not flowered by day 321 when plants were taken to the greenhouse to avoid frost.

Results are given for caging D. heterocarpon 6105 and 6102 in Gainesville in 1987 in Table 2.9. Three of eight plants of 6105 and three of six plants of 6102 were enclosed in individual screened cages. All plants of both lines, both caged and uncaged, produced seed. Total seed production varied from 0.71 gms for an uncaged plant of line 6102 to 26.32 gms of seed produced by a caged plant of line 6105.

Seed set on Desmodium in the greenhouse is presented in Table 2.2. 6150, U.F. 28, U.F. 160, hybrid 83-4-3, and hybrid 83-4-2 set no seed on flowers that were not tripped manually. Percentage pod set on unmanipulated flowers of other lines ranged from 0% to 25%. On five lines which were self-pollinated manually, pod set ranged from 11% to 73% whereas seven lines which were cross-pollinated had pod set of 08% to 57%.

Table 2.8. Frequency distributions for flowering dates of F_1 and F_2 191 in Gainesville, Florida in 1987.

	Days of year										
Line	256	262	276	288	293	301	307	321	n	$\bar{x}$	o^2
28							0[a]		0		
6105	1	4	4						9	267.5	7.8
191 F^1					1	2			3	298.3	3.8
191 F^2			4	7	10	9	3	7[b]	33	293.3	14.54

[a] No plants had flowered by day 307 in Gainesville in 1986, at which date frost occurred.

[b] Seven plants had not yet flowered by day 321 when frost occurred in 1987. Plants that have not flowered by this date usually cannot produce seed before frost in Gainesville.

Table 2.9. *Desmodium heterocarpon* caged and uncaged seed production in Gainesville, Florida in 1987.

Line Number	Plant Number	Caged	Seed Weight[a]
			---gm---
U. F. 6105	1	yes	2.78
D. heterocarpon	2	yes	26.32
	3	yes	19.95
	4	no	8.00
	5	no	1.35
	6	no	1.69
	7	no	1.38
	8	no	5.46
IRFL 6102	1	yes	7.9
D. heterocarpon	2	yes	10.05
	3	yes	7.80
	4	no	.71
	5	no	3.89
	6	no	1.05

[a] Mean seed weight between caged and uncaged was not different ($P>0.05$) for 6105 and 6102.

Discussion

Interest in percentage outcrossing versus selfing in D. heterocarpon and D. ovalifolium was generated by observed differences in pod set in the greenhouse. Desmodium heterocarpon appeared to set profuse amounts of pods with no manipulation, whereas D. ovalifolium-type lines set few pods if not manipulated. Caging experiments were conducted in the field to determine whether either species needed flower tripping by insects or cross-pollination to set pods. Both species appear to be photoperiod sensitive and triggered to flower by shortening daylength. This photoperiodism complicated caging experiments by restricting the number of lines available to be caged. The variation in daylength in Cameroon (latitude 3°N) is so slight that during the time available for caging studies during the summer of 1986, only two D. ovalifolium lines flowered. These two lines (3325 and U. F. 146) gave uniform results. Caged plants produced no pods while uncaged plants produced pods containing seed. It is interesting to note that flowering response was not uniform within these two lines. Each line had one plant that did not flower. Presence of immature seed in pods was noted as an indication of successful pollination. Pods were harvested before seeds were mature to allow analysis of results during the time allowed in Cameroon. An additional line of D. ovalifolium was caged in Gainesville, Florida.

This line (CIAT 13110) gave results similar to caging results from Cameroon, although caged plants did produce a small number of pods containing seed. Two uncaged plants of this line produced large numbers of pods (>2,000) per plant. A T test for homogeneity does not show caged versus uncaged results to be different (P,0.05), but this is most likely an artifact due to high variability within the uncaged plants where one plant produced only 117 pods. Two D. heterocarpon that most resemble the agronomic description of D. hetercarpon ssp. heterocarpon var. heterocarpon (6105 and 6102) were caged in Gainesville. The results were measured in seed weight, because the high number of pods set precluded counting individual pods. Caged and uncaged plants produced large numbers of seed. Large amounts of seed were produced by the caged plants. This may be due to increased humidity within the cages, or lower temperatures. Rotar and Chow (1971) have reported that high humidity and low temperatures increased success for controlled crosses between D. intortum, D. sandwicense, and D. uncinatum. Two lines of D. heterocarpon ssp. heterocarpon var. strigosum were caged and gave mixed results. 6155 set high numbers of pods whether plants were caged or not. 6106 gave signifcantly different results with caged plants producing very few seed. These plants were caged late in the year after they had already been flowering for some time. All flowers and seeds were removed from the plants at the start of this experiment, but plants tended to resume vegetative

regrowth rather than flowering. The microclimate produced by the cages may have affected flowering.

Results from caging experiments indicated that D. ovalifolium required some manipulation of flowers denied it under caged conditions. Either the plant required insect cross-pollination, or it required insects to trip the flowers. Desmodium heterocarpon types appeared to set seed regardless of whether the plants were caged or not, indicating that flower manipulation and cross-pollination were not necessary for seed set. These conclusions are drawn from a small sample, but indicate that a distinction between D. ovalifolium and D. heterocarpon may be the need for flower manipulation or outcrossing in D. ovalifolium and not in D. heterocarpon.

Casual observations of desmodium in the greenhouse indicated that 6102 and 6105, both D. heterocarpon, set large amounts of pods without manipulation of the flower. Furthermore, the flowers on these two lines self-tripped early in the morning. This was in contrast to the D. ovalifolium whose flowers remained untripped until late in the day. Neither D. ovalifolium line produced seeds from tagged flowers that were not tripped, nor did two hybrids (83-4-3 and 83-4-2). The remaining three hybrids each produced one pod, indicating that seed production from untripped flowers was extremely low. In an attempt to determine if tripping and self-pollinating would increase pod set, three lines were tripped and selfed at 10:00 A.M.

each morning. Here the distinction between D. ovalifolium and D. heterocarpon breaks down. Accession 6150, although morphologically neither a typical D. heterocarpon or D. ovalifolium type, is classified as D. heterocarpon ssp. heterocarpon var. heterocarpon. It has white flowers that do not self-trip, small non-pubescent leaves, and a shrublike growth habit. It required tripping in the greenhouse for pod set. When tripped, pod set increased 11%. Tripping and self pollinating the D. ovalifolium lines increased pod set to by 60 to 73%. The same three lines were tripped and cross pollinated but this did not improve pod set. These results indicate that the D. ovalifolium lines are not obligate outcrossers, but that flowers of these lines do require manipulation for pods set.

Marked leaf has been used by many researchers as an indication of successful cross pollination in crosses that involve maternal unmarked leaf and paternal marked leaf parents. Segregation data for the F_2 generation of such a cross (U. F. 28 unmarked leaf D. ovalifolium x 6150 marked leaf D. heterocarpon) gave a 9:7 ratio for marked leaves. The marked leaf parent when self-pollinated did not appear to segregate for marked leaf indicating that the accession was homozygous for the trait. In several other crosses made by Dr. K. H. Quesenberry, anytime the F_1 appeared to be morphologically intermediate between a unmarked-leaf D. ovalifolium parent and a marked-leaf D. heterocarpon parent, the F_1 has always had marked leaves.

Another morphological marker used to indicate a successful cross is flower color. The data fit the 3:1 purple:white flower ratio expected for a single gene dominant trait. Usefulness of this marker is restricted by the limited number of white flowered lines in the U. F. germplasm collection although the white flower trait could be incorporated into more lines.

Several accessions of Desmodium, particularly D. ovalifolium types, flower too late in the year to produce seed before frost. Wide variability exists within the germplasm for date of flower. Flowering seems to be in response to shortening photoperiod. Table 2.8. shows the spread of flower date for the segregating F_2 population of a cross made between lines with widely divergent flower dates (U. F. 28 x 6105). U. F. 28 flowered so late in the year that the F_1 cross was made in the protected environment of a greenhouse during the winter. IRFL 6105, on the other hand, flowered early in the year (day 262) and produced a heavy seed set before frost. The F_1 of cross U.F. 28 x 6105 flowered on day 298 which was approximately the median between the two parents. The bloom dates for the F_2 population were from day 276 to later than day 307. The mode for the F_2 bloom date was the same as the bloom date for the F_1 plants, day 298. This variation in bloom date indicates that change in bloom date should be responsive to manipulation through controlled crosses and selection.

Ohashi (1973) has proposed a taxonomic key for the species D. heterocarpon that divided the species into two subspecies, heterocarpon and angustifolium. His most readily apparent distinction was the leaf length to width ratio for subspecies angustifolium which was given as 3 - 6.5 as opposed to subspecies heterocarpon which he described as having leaf length to width ratios of less than 3. Three lines were classified as D. heterocarpon subspecies angustifolium among the lines tested. 6125 and 6123 had leaf length to width averages that were greater than 3 when grown both in Cameroon and in Florida. 6143 had leaf length to width averages (0.8 in Cameroon and 1.4 in Florida) outside of the specifications for subspecies angustifolium. Ohashi (1973) remarked that D. heterocarpon was one of the most highly polymorphic species. This exception to leaf length to width ratio was either an example of morphological variation which in this case extended outside the range of the subspecies or indicates this was an incorrectly identified line.

No clear-cut differences existed between D. heterocarpon and D. ovalifolium lines in plant height or plant width, although D. ovalifolium lines tended to be grouped among the shorter lines. Leaf mark also did not strictly divide along lines of D. heterocarpon and D. ovalifolium. Most D. ovalifolium lines had unmarked leaves, although 3664 had marked leaves. Most D. heterocarpon ssp. heterocarpon var. heterocarpon accessions had marked leaves, although

some, as in line 6108, did not. Thus presence or absence of marked leaves is not a steadfast indicator of whether a line is D. ovalifolium or D. heterocarpon, if these lines are correctly classified.

CHAPTER III
DESMODIUM HYBRID IDENTIFICATION USING ISOZYME ELECTROPHORESIS

The genus Desmodium is pan-tropical with various species found in sub-tropical and tropical areas of Southeast Asia, Africa, and Central America (Imrie et al., 1983). Interest in Desmodium heterocarpon (L) DC. as a tropical forage legume for Florida results from the fact that the species is able to fix nitrogen and that it is the best adapted perennial pasture legume for summers in Florida (Pitman and Kretschmer, 1984). Taxonomically D. heterocarpon has been considered to include Desmodium ovalifolium Wall. by some but not all researchers (Ohashi, 1973; Schultze-Kraft, personal communication). The cultivar 'Florida' carpon desmodium, Desmodium heterocarpon, was selected from plant introduction evaluations and released in 1979 (Kretschmer et al., 1979). 'Florida' has shown several desirable attributes including perenniality, good seed production, and some tolerance to wet soils, but it has been shown susceptible to root-knot nematode (Kretschmer et al. 1980). Initial screening for nematode resistance in lines of Desmodium has shown that some lines of the D. ovalifolium type have root-knot nematode resistance, a valuable asset in Florida soils (Quesenberry and Dunn, 1987). Ideally, breeding for nematode resistance in Desmodium could

combine the root-knot resistance of the D. ovalifolium types with the agronomic characters of earlier flowering and cold hardiness of the released cultivar Florida.

Most D. heterocarpon have marked leaves whereas most D. ovalifolium types have unmarked leaves. The leaf-mark in D. uncinatum and D. intortum have been shown to be controlled by dominant genes (Chow and Crowder, 1973). Use of this leaf mark to verify hybrids requires that crosses be made between female D. ovalifolium parents and male D. heterocarpon parents. At times it may be desirable to make the reciprocal cross or to cross two parental lines both of which have marked leaves. Thus identification of other morphological or other type markers are needed. An alternative method of identifying parents and hybrids would be the use of isozyme markers to indicate successful hybrids of D. heterocarpon x D. ovalifolium. Isozyme markers have been used to identify hybrids in bananas (Musa spp.) (Jarret, 1987), peach [Prunus persica L.(Batsch)] (Durham et al, 1987), and papaya (Carica papaya L., Carica cauliflora Jacq. and hybrids) (Moore and Litz, 1984). Isozyme markers have also allowed researchers to distinguish between morphologically similar lines within a germplasm collection of bananas (Jarret, 1987), alfalfa (Medicago sativa L.) (Quiros, 1980), Lolium perenne (L.) (Hayward and McAdam, 1977), and cassava (Manihot esculenta Crantz) (Ramirez et al., 1987). Isozyme markers have been used to verify hybrids of Desmodium intortum (Mill.) Urb. x Desmodium

uncinatum (Jacq.) DC., and Desmodium sandwicense E. May. x D. intortum (Chow and Crowder, 1973), to detect the presence of Desmodium sandwicense genes within D. intortum 'Greenleaf' (Imrie and Blogg, 1982) and, with Desmodium ovalifolium, to distinguish lines of a germplasm collection (Hussain et al, 1987). The primary objective of this research was : (1) to identify an enzyme system which showed isozyme variability between D. hetercarpon and D. ovalifolium and, (2) to evaluate the use of such a system to distinguish F_1 hybrids from their parental types.

Materials and Methods

Experiment 1: Desmodium ovalifolium lines U. F. 28 and U. F. 160 and D. heterocarpon lines 6102 and 6105 were screened for activity using 24 stains and 6 buffers (Table 3.1. and Table 3.2.). Leaf tissue came from mature, greenhouse grown plants and was selected from the eight most recent, fully expanded leaves on any branch. Leaf tissue was prepared according to three methods. In the first method, a leaf was placed on a glass plate, a 6 x 6 mm section of Whatmann #1 filter paper (wick) placed over it, and the head of a pestle rolled over the paper squeezing cell sap from the leaf into the paper. In the second method leaf tissue was ground in a mortar with a pestle in 1% glutathion in 0.1 M Tris HCl at pH 7. The third method was identical to the second, except that 0.015 gm of polyvinyl

Table 3.1. Enzyme activity stains and buffer systems investigated for the detection of isozyme banding patterns from leaf extracts of D. heterocarpon and D. ovalifolium.

Enzyme system	Reference	Buffer system
Acid phosphatase (EC 3.1.3.2)	b	TC
Alcohol dehydrogenase (EC 1.1.1.1)	c	H 6.3[a]
Ascorbate oxidase(EC1.10.3.3)	c	TB,LBTC[a],K
Catalase (EC 1.11.1.6)	c	TB, LBTC, K
Diaphorase (EC 1.6.4.3)	c	H
Esterase (EC 3.1.1.2)	b	TB, LBTC, K
Formate dehydrogenase (EC 1.2.1.2)	d	H
beta-D-Galactosidase (EC 3.2.1.23)	c	TB, LBTC, K
Glucose-6-phosphate dehydrogenase (EC 1.1.1.49)	c	TB, LBTC
Glutamate oxaloacetate transaminase (EC 2.6.1.1)	b	TB, LBTC, K
Glutathione reductase (EC 1.6.4.2)	c	TB[a],K
Hexokinase (EC 2.7.1.1)	e	TC
Isocitrate dehydrogenase (EC 1.1.1.42)	b	LBTC, TC
Leucine aminopeptidase (EC 3.4.11.1)	b	H
Malate dehydrogenase (EC 1.1.1.37)	b	LBTC, H 6.3
Malic enzyme (EC 1.1.4.0)	b	TC
Peroxidase (EC 1.11.1.7)	b	TB, LBTC[a], K[a], TC[a]
Phosphoglucomutase (EC 2.7.5.1)	b	H, LBTC, H 6.3[a]
6-Phosphogluconate dehydrogenase (EC 1.1.1.44)	c	H, LBTC, H 6.3
Phosphohexose isomerase (EC5.3.1.9)	b	TB[a],LBTC, K[a], H 6.3[a]
Shikimate dehydrogenase (EC 1.1.1.25)	c	TB, LBTC, K
Superoxide dismutase (EC 1.15.1.1)	b	TB[a], LBTC, K
Urease (EC 3.5.1.5)	c	TB, K

[a] Buffer system with visible results
[b] Durham (1986)
[c] Vallejos (1983)
[d] Wendel and Parks (1982)
[e] Shaw and Prasad (1970)

Table 3.2. Electrophoretic buffer systems used for analysis of D. heterocapon and D. ovalifolium leaf and root extracts.

Buffer system[a]	Electrode buffer	Gel buffer[b]
H	0.065 M histidine 0.02 M citric acid pH 5.7 with citric acid	0.009 M histidine 0.003M citric acid pH 5.7 with citric acid
H 6.3	0.065 M histidine 0.02 M citric acid pH 6.3 with citric acid	0.009 M histidine 0.003 M citric acid pH 6.3 with citric acid
LBTC	0.016 M LiOH 0.192 M boric acid pH 7.2	0.0016 M LiOH 0.019 M boric acid 0.007 M citric acid 0.046 M trizma base pH 7.7
TB	0.038 M trizma base 0.002 M citric acid pH 8.6	0.03 M boric acid pH 8.5
TC	0.05 M trizma base 0.016 M citric acid pH to 7.0 with citric acid	0.017 M trizma base 0.005 M citric acid pH to 7.0 with citric acid
K	0.18 M trizma base 0.1 M boric acid 0.004 M Na_2EDTA pH 8.6	0.045 M trizma base 0.025 M boric acid 0.001 M Na_2EDTA pH 8.6

a H= Histidine citrate (pH 5.7), Histidine citrate (pH 6.3), LBTC=Lithium borate/tris citrate, TB=Tris/borate, TC=Tris citrate (Durham 1986).

b 10 mg NADP was added to buffer in cathodal tank and gel buffer of systems that included analysis for enzymes that required NADP as a cofactor.

polypyrrolidone (PVPP) was added.

Starch gels (11%) were prepared by heating 21 grams Sigma and 10 grams of Connaught starch in 300 mls of the appropriate buffer, degassed, and poured into 19 x 19 cm gel molds to a thickness of 0.5 cm. NADP, when used as a cofactor, was added during the heating process. Each gel contained 12 wicks representing the 12 combinations of four lines prepared each of 3 ways. As a result of this screening, stains for peroxidase and phosphohexose isomerase were chosen for more extensive trials.

Experiment 2: Leaf tissue from five parental lines and five hybrids (Table 3.3.) were evaluated for response to peroxidase and phosphohexose isomerase. Leaf tissue (2 cm x 1 cm) was taken from the most recent, fully expanded leaf. Root tissue (5 cm long) was taken from approximately five terminal root sections, although older roots were used when new growth was not available. Five plants of each parental line, and one of each F_1 hybrid, were evaluated on each of three different days on three different gels. Leaf tissue was macerated in five drops of Tris HCl (pH 7.8 0.1M). Root tissue was macerated in five drops of Tris HCl (pH 7.8 0.1M) with the addition of 2mg PVPP. Crude extract was absorbed onto 6 x 6 mm filter paper wicks (Whatmann #1) and inserted into a slit in the gel.

Starch gels (8.3%) were prepared as above except that only 25 grams Sigma starch and no Connaught starch was used. Initially several concentrations of starch ranging

Table 3.3. *Desmodium ovalifolium* and *D. heterocarpon* lines and hybrids examined for isozyme markers.

Line #	Species
IRFL 6102	*D. heterocarpon*
U.F. 28	*D. ovalifolium*
IRFL 6105	*D. heterocarpon*
U.F. 160	*D. ovalifolium*
IRFL 6106	*D. heterocarpon*
Hybrid	
191 F_1 (28 x 6105)	
195 F_1 (160 x 6102)	
83-4-3 F_1 (160 x 6106)	
83-4-2 F_1 (160 x 6106)	
83-3-3 F_1 (160 x 6106)	

from 25 to 30 grams were used but 25 grams (8.3%) produced gels that were easier to make and handle than 10% gels, and gave equivalent results.

Leaf tissue was analyzed for anodal peroxidase using Tris-citrate buffer. Root tissue was analyzed for cathodal peroxidase and phosphohexose isomerase using K buffer (Table 3.2.). All gels were run at constant 250 volts, and terminated when Bromo methyl blue marker indicated the anodal front had moved 8 cm. Gels were sliced into three horizontal sections, with the top section thrown away. The middle and bottom section of the cathodal end of K-buffer gels were stained for peroxidase activity (PER: E.C. 1.11.1.7) using the method of Durham et al. (1987). The middle and bottom section of the anodal end was stained for phosohexoseisomerase (PHI: Glucophosphate isomerse, E.C. 5.3.1.9) using the method of Durham et al. (1987). The middle and bottom section of anodal ends of tris citrate (TC, Table 3.2.) gels were stained for peoxidase.

Results

Results of an overall stain evaluation program are given in Table 3.1. Many staining systems were tried only once. Those that gave no visible results were not repeated. All buffer systems marked with a superscript [a] were systems that produced visible banding.

A zymogram of cathodal peroxidase patterns for D. ovalifolium U.F. 28, D. heterocarpon 6105, and their F_1 191 is given in Fig. 3.1. 6105 had two bands of activity: a slow band extending from R_f 0.10 to 0.13. and a fast band extending from R_f 0.22 to 0.26. U.F. 28 also had two bands of activity: a slow band extending from R_f 0.18 to 0.21 and a fast band from R_f 0.23 to 0.27. Hybrid 191 had three bands of activity: a slow one from R_f 0.11 to 0.15, another slow one from R_f 0.17 to 0.20, and a fast one extending from R_f 0.23 to 0.28.

A zymogram of cathodal peroxidase patterns for D. ovalifolium U.F. 160, D. heterocarpon 6102, and their F_1 hybrid 195 is given in Fig. 3.2. U.F. 160 had two bands of activity: a slow one extending from R_f 0.18 to 0.22 and a fast one extending from R_f 0.26 to 0.30. 6102 had two bands of activity: a slow one extending from R_f 0.12 to 0.18 and a fast one extending from R_f 0.27 to 0.32. Similar to hybrid 191, hybrid 195 had three bands of activity: a slow band extending from R_f 0.12 to 0.17, another slow band extending from R_f 0.20 to 0.25, and a fast band extending from R_f 0.28 to 0.33. A photograph of cathodal peroxidase is shown in Fig. 3.3.

A zymogram of anodal PHI for root tissue for D. ovalifolium U.F. 160, D. heterocarpon 6102, and 160 x 6102 hybrid 195 is given in Fig. 3.4. U.F. 160 has two bands of activity of which the slow one, R_f 0.18 to 0.22, was consistent. The fast band extended from R_f 0.37 to 0.46,

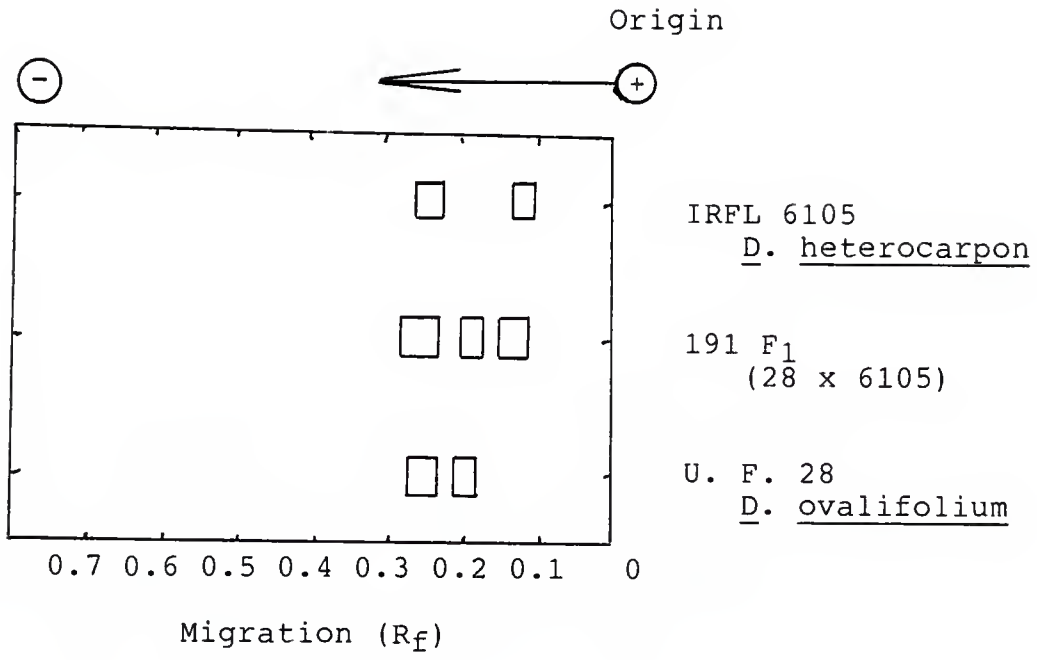


Figure 3.1. Illustrative drawing of cathodal peroxidase isozyme banding patterns for U. F. 28, 6105, and their hybrid.

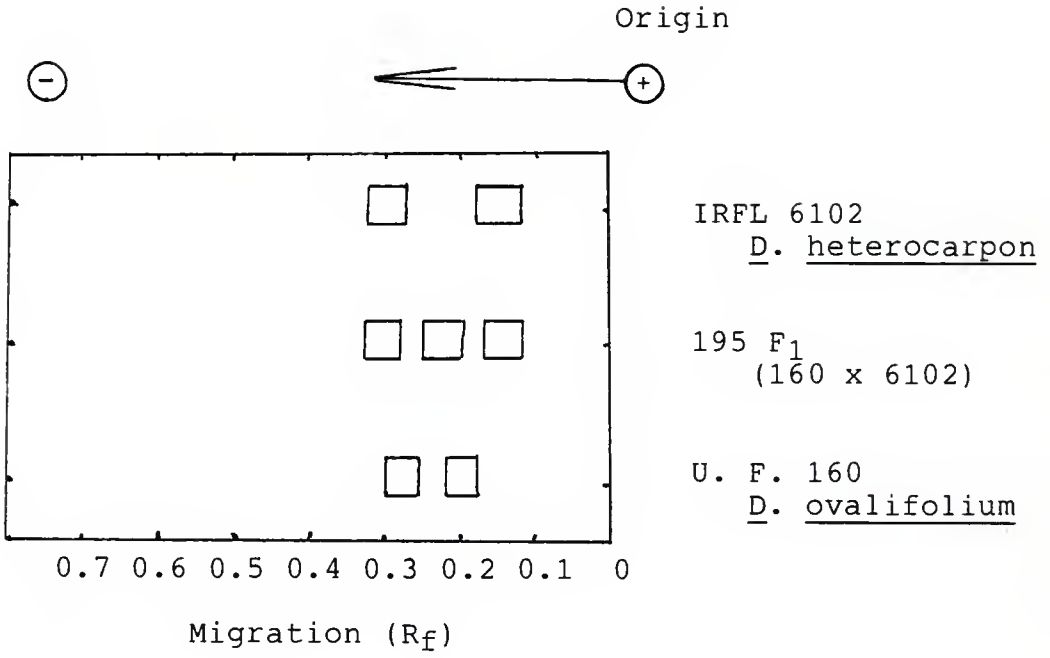


Figure 3.2. Illustrative drawing of cathodal peroxidase isozyme banding patterns for 6102, U. F. 160, and their hybrid.

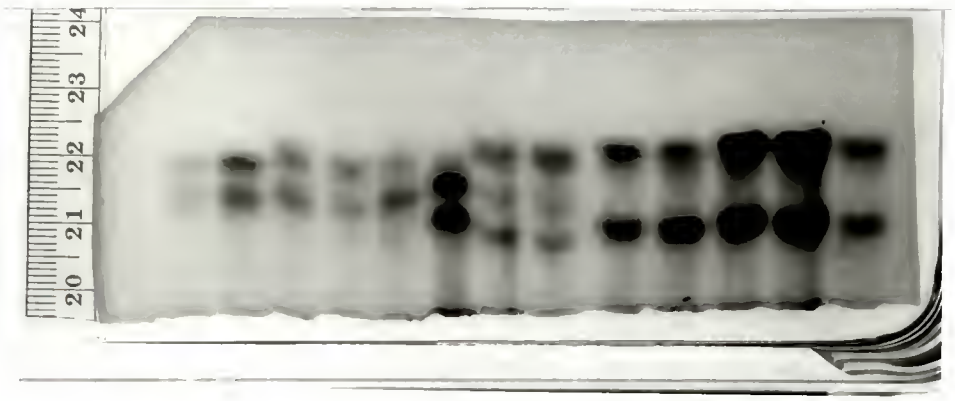


Figure 3.3. Cathodal peroxidase banding patterns from left to right, U.F. 160 (five plants in five lanes), hybrid 160 x 6102 (material from one plant in each of three lanes, each prepared with a different extraction buffer),and 6102 (five plants in five lanes).

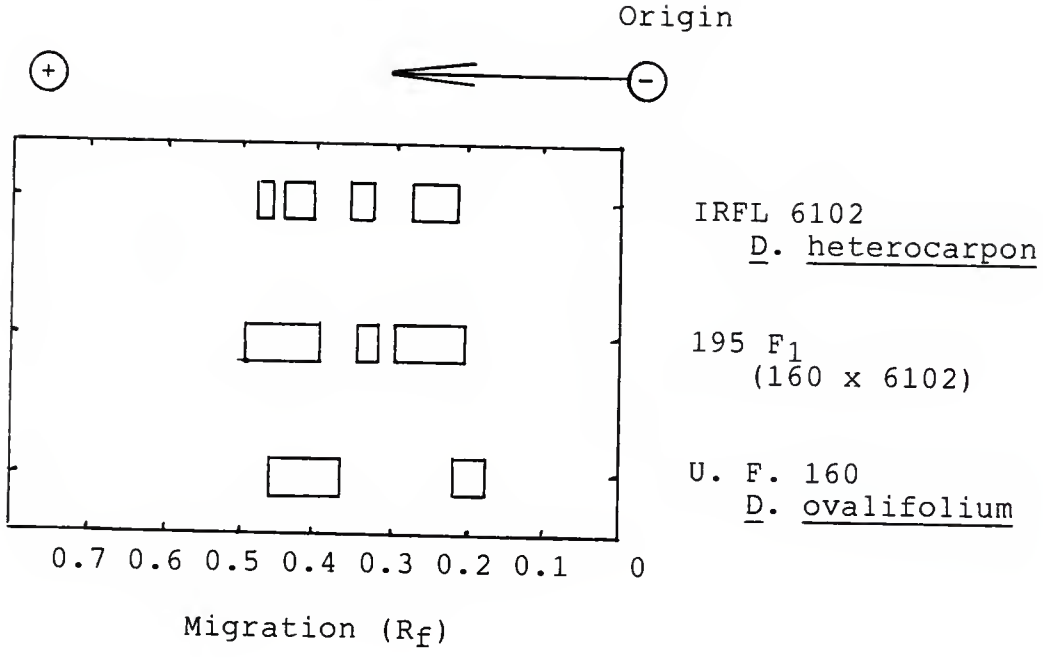


Figure 3.4. Illustrative drawing of anodal PHI isozyme banding patterns for IRFL 6102, U. F. 160, and their hybrid.

but was variable, as were other less distinct areas of staining. 6102 has four bands of activity with the slow one extending from R_f 0.22 to 0.28. The other three bands extended from R_f 0.33 to 0.36, 0.41 to 0.45, and 0.46 to 0.48. The hybrid 195 has three bands of activity of which the slow one extends from R_f 0.21 to 0.30 with two others from R_f 0.32 to 0.35 and from 0.40 to 0.50.

A zymogram of anodal PHI for root tissue for D. ovalifolium U.F. 160, D. heterocarpon 6106, and 160 x 6106 hybrid 83-4-2 is shown in Fig. 3.5. 6106 had four bands of activity with three of those bands close together in the slow region. These bands are at R_f 0.18 to 0.23, 0.25 to 0.27, and 0.30 to 0.33. A faster band consistently showed up between R_f 0.42 and 0.48. U.F. 160 is the same as in Fig. 2.4. Hybrid 83-4-2 has three bands of activity with the slow one extending from R_f 0.18 to 0.27. Two other bands extended from R_f 0.31 to 0.40, and from 0.45 to 0.50.

A composite photograph of anodal PHI is shown in Fig. 3.6. with five lanes of U.F. 160, one lane of 195, five lanes of 6102, one lane of 83-4-2, and five lanes of 6106. Root tissue from a different plant was used to load each lane.

A zymogram of anodal peroxidase for D. ovalifolium U.F. 160, D. heterocarpon 6106, and 160 x 6106 hybrids 83-4-2, 83-4-3, and 83-3-3 is given in Fig. 3.7. U.F. 160 had two bands of activity: a slow one extending from R_f

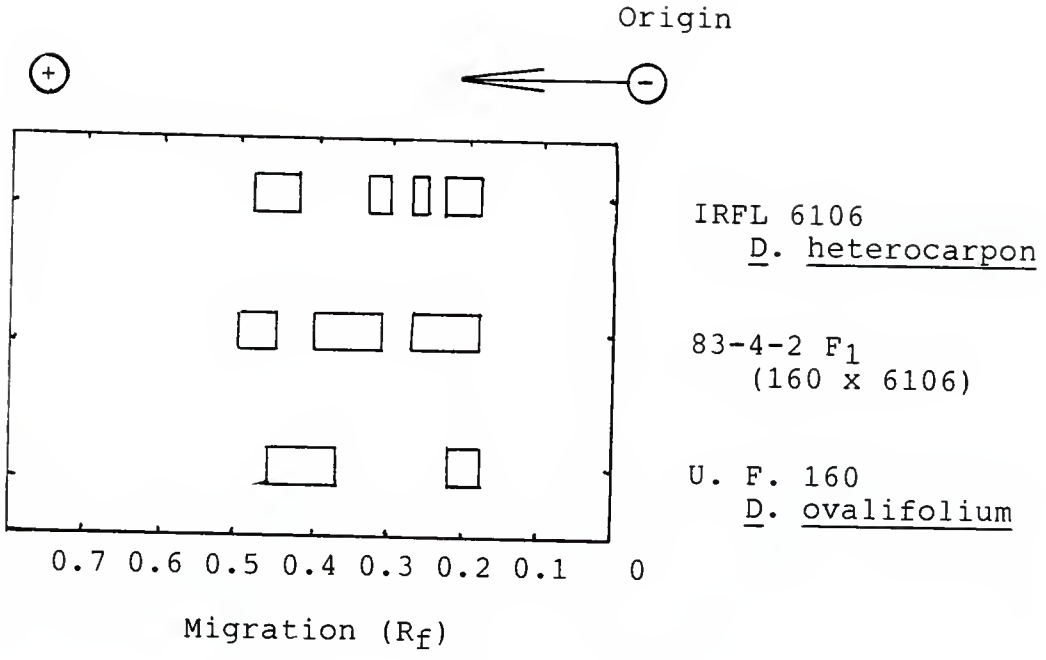


Figure 3.5. Illustrative drawing of anodal PHI isozyme banding patterns for U. F. 160, IRFL 6106, and their hybrid.

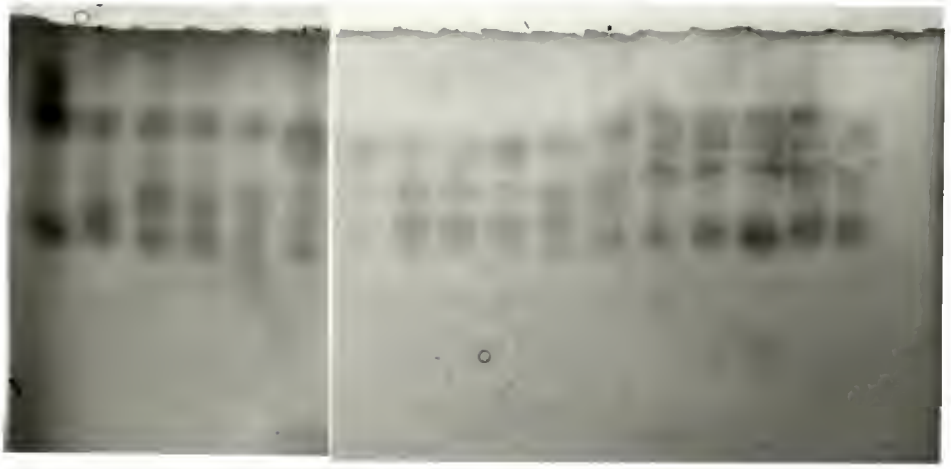


Figure 3.6. Composite photograph of isozyme banding patterns observed for anodal PHI from left to right, U.F. 160 (five plants in five lanes), hybrid 195 (U.F.160 x 6102), 6102 (five plants in five lanes), hybrid 83-4-2 (U.F.160 x 6106), 6106 (five plants in five lanes).

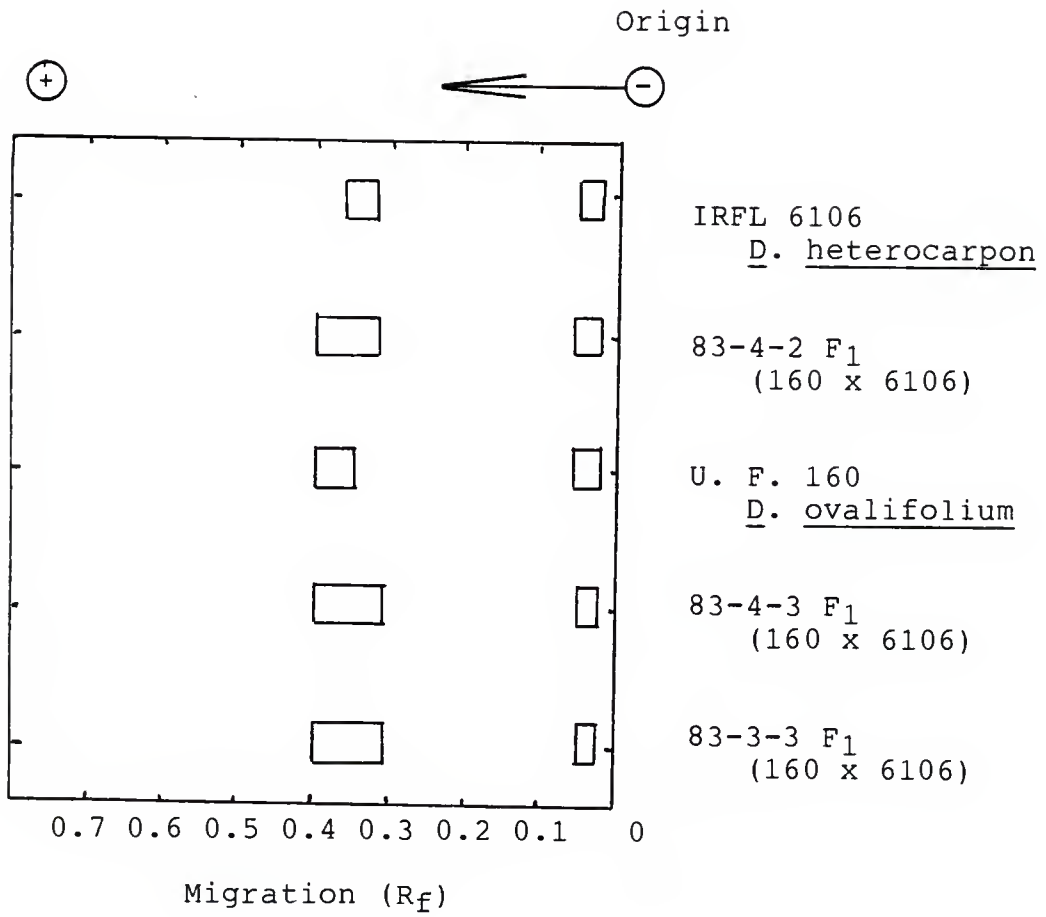


Figure 3.7. Illustrative drawing of anodal peroxidase isozyme banding patterns for U. F. 160, IRFL 6106, and their hybrids.

0.02 to 0.06 and a fast one extending from R_f 0.35 to 0.40. 6106 had two bands of activity: a slow one extending from R_f 0.02 to 0.05 and a fast one extending from 0.32 to 0.36. Hybrid 83-4-2 had two bands of activity: a slow one extending from 0.02 to 0.06 and a fast one extending from 0.32 to 0.40. Hybrid 83-4-3 had two bands of activity: a slow band extending from R_f 0.02 to 0.05 and a fast one extending from 0.31 to 0.40. Hybrid 83-3-3 had two bands of activity: a slow one extending from 0.02 to 0.05 and a fast one extending from 0.31 to 0.40.

Figure 3.8. shows a representative photograph of anodal peroxidase for U.F. 160 (five lanes), 160 x 6106 hybrid 83-4-2 (one lane), and 6106 (five lanes). Leaf tissue from a different plant was used to load each lane.

Discussion

Banding patterns for D. heterocarpon 6105, D. ovalifolium U.F. 28, and 6105 x 28 hybrid 191 were distinct for each line. The F_1 hybrid had three bands while each parent had two bands. One of the two slower bands migrated at the same speed as the slow band of U.F. 28. The other slow band migrated at the same speed as the slow band of 6105. The fast band in the F_1 hybrid migrated at the same speed as the fast band of each parent. This banding pattern could be explained by gene action of two loci. The locus for the fast band is homozygous and the same for each

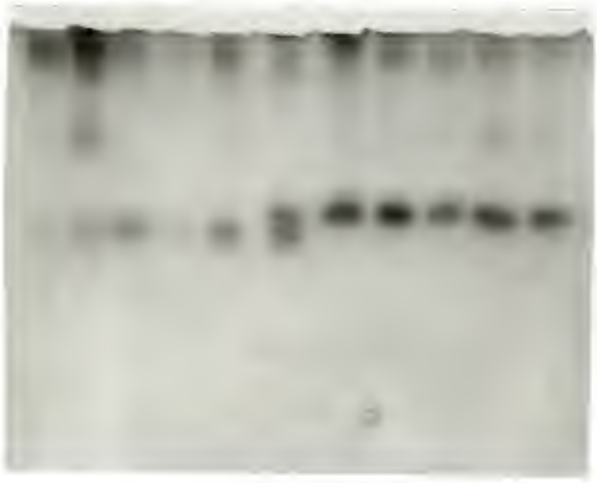


Figure 3.8. Banding patterns observed for anodal peroxidase from left to right, five lanes U.F. 160 (five different plants), hybrid 83-4-2 (U.F. 160 x 6106), five lanes of 6106 (five different plants).

for 6105 is probably homozygous as is the slow band R_f 0.18 to 0.21 for U.F. 28. The hybrid locus is heterozygous for this trait with bands at R_f 0.11 to 0.15 and R_f 0.17 to 0.20, that is bands roughly equivalent to the two parental slow bands.

Banding patterns for D. heterocarpon 6102, D. ovalifolium U.F. 160, and 6102 x 160 hybrid 195 were distinct for each line. It should be noted that cathodal R_f values were calculated as if the cathodal front had moved 8 cm while the anodal front was moving 8 cm. The F_1 hybrid had three bands while each parent had two bands. The three hybrid bands appeared to be two slow bands, each of which was similar to one of the parent slow bands, and one fast band identical to the fast band of each parent. The most likely interpretation of this banding pattern is similar to 191 above. The locus for the fast band is homozygous and the same for each parent and the hybrid. The locus for the slow band R_f 0.12 - 0.18 for 6102 is probably homozygous as is the slow band R_f 0.18 to 0.22 for U.F. 28. The hybrid locus is heterozygous for this trait with bands at R_f 0.12 to 0.17 and R_f 0.20 to 0.25, that is bands roughly equivalent to the two parental slow bands. Parental D. ovalifolium lines 160 and 28 are very similar in morphology, as are parental D. heterocarpon lines 6102 and 6105, therefore it is not surprising that there hybrids 191 and 195 are similar in morphology and in isozyme marker patterns.

Desmodium ovalifolium U.F. 160, D. heterocarpon 6102, and their hybrid 195 each showed distinct banding for root material stained for PHI isozymes. U.F. 160 showed two bands of activity, 6102 four bands, and the hybrid three bands. The bands of interest for distinquishing parental lines and their hybrids from one another were the slow bands. The slow band in U.F. 160 extended from R_f 0.18 to 0.22 while the slow band in 6102 extended from R_f 0.22 to 0.28. The hybrid slow band was larger and somewhat intermediate, extending from R_f 0.21 to 0.30. The uniqueness of the hybrid band makes it adequate for hybrid verification without determining the number of bands that actually compose it.

Desmodium ovalifolium U.F. 160, D. heterocarpon 6106, and the hybrid 83-4-2 each showed distinct banding for root material stained for PHI. U.F. 160 showed bands as described above. The bands of interest for purposes of distinguishing parental lines from each other and their hybrid were the slow bands. The slow band in U.F. 160 was as listed above while the slow banding area in 6106 was composed of three bands that extended from R_f 0.18 to 0.23, 0.25 to 0.27, and 0.30 to 0.33. The hybrid slow band was larger and somewhat intermediate, extending from R_f 0.18 to 0.27. This banding pattern was sufficient for verification purposes to conclude that the parents and hybrids were different.

The banding pattern for anodal peroxidase showed two bands for D. ovalifolium U.F. 160, D. heterocarpon 6106, and the hybrids 83-4-2, 83-4-3, 83-3-3 all of which were 160 x 6106 F_1 hybrids. The slow band was the same for each species and hybrid covering an area roughly R_f 0.02 to 0.06. The fast band in U.F. 160 migrated at R_f 0.35 to 0.40 and in 6106 migrated at R_f 0.32 to 0.36. In the hybrids, the fast band covered the area of both parental bands extending from roughly R_f 0.31 to 0.40 and probably is two bands and could indicate heterozygosity for a monomer at that locus. However, the gene action is speculative. This staining system is capable of verifying hybrids.

Isozyme electrophoresis of Desmodium using starch gels is a straight forward procedure that can be used to verify a large number of hybrids in a short period of time. Crosses with D. heterocarpon and D. ovalifolium could be made in either direction without concern for the presence of a dominant morphological marker in the male parent. This system could also be used to delineate gene action by looking at segregation ratios in the F_2 populations. Additional work needs to be done using a large population of D. heterocarpon and D. ovalifolium plants to verify whether isozyme markers can distinquish among lines in each species.

CHAPTER IV
CYTOLOGY OF *DESMODIUM HETEROCARPON* AND *DESMODIUM OVALIFOLIUM*

The haploid chromosome number of 11 was reported for North American East Coast *Desmodium* (24 species) by Young (1939-1940). Several workers have observed that difficulty in obtaining differential staining between the cytoplasm and the chromosomes was a serious impediment to cytological work (Young, 1939-1940; Rotar and Urata, 1967; Schifino, 1983). Schifino (1983) looked at four species of *Desmodium* and reported that the 2n number was 22 for all four species. She examined two morphologically different forms of *D. uncinatum* (L.) DC. and found that their similar chromosome number supported Oliveira's contention that they are one species.

Failure of chromosome pairing was noted in *Desmodium intortum* (Mill.) Urb. x *Desmodium sandwicense* E. Mey F_2 hybrids (Hacker, 1968). Hacker reported that one of the F_2 plants had laggards in 25% of the cells studied and showed 78% pollen stainability. However, Hacker's pollen stainability varied within plant, from flower to flower, and even from anther to anther. Hacker also referred to the possible formation of multicellular tetrads in *Desmodium* instead of micronuclei as a result of delayed cytokinesis.

'Florida' carpon [Desmodium heterocarpon (L) DC.]) was released for use in Florida in 1979 (Kretschmer et al., 1979). 'Florida' carpon has perenniated in central Florida, has good seed production, and showed tolerance to wet soils, but was susceptible to root-knot nematode (Kretschmer et al., 1979). Initial screening of Desmodium ssp. germplasm indicated that D. heterocarpon (L) DC. was less resistant to Meloidogyne arenaria race 1 (Neal) Chitwood, M. incognita race 3 (Kofoid and White) Chitwood, and M. javanica (Treub) Chitwood than was D. ovalifolium (Quesenberry et al., 1986). Attempts to hybridize D. ovalifolium and D. heterocarpon in the greenhouse resulted in few successful crosses (Quesenberry and McKellar, unpublished results). These two species are taxonomically considered one species by Ohashi (1973), therefore this difficulty in making crosses was not anticipated. The objectives of this research were twofold: (1) to evaluate D. heterocarpon and D. ovalifolium pollen viability, and (2) to characterize meiotic regularity in parent lines of D. heterocarpon and D. ovalifolium and their hybrids.

Materials and Methods

Mature plants of D. heterocarpon, D. ovalifolium, and their hybrids were maintained in the greenhouse at University of Florida. Seed sources of parental lines were the Centro Internacinal de Agriculture Tropical (CIAT)

and Dr. A. E. Kretschmer, Jr., Agricultural Research and Extension Center, Fort Pierce, Florida (Table 4.1.). Desmodium hybrids evaluated in this study are also described in Table 4.1. Some, but not all, CIAT D. heterocarpon accessions were taxonomically classified as to species, subspecies, and variety. When available, this classification was followed. Other D. heterocarpon lines were taxonomically ranked by CIAT only to species. Desmodium ovalifolium and D. heterocarpon not ranked by CIAT were listed as D. ovalifolium or D. heterocarpon based on gross morphological characters. All plant material was one year old or older.

Bud material was collected in the form of entire racemes before buds were visible between the bracts. Racemes were placed immediately in 95% ethanol:chloroform: glacial acetic acid (6:3:1, v:v:v) and refrigerated for 24 hours. Racemes were then placed in 70% ethanol and refrigerated until use, usually within two weeks and always within one month.

Cytological examination of chromatin and pollen tetrads was done with individual buds removed from the raceme under a dissecting scope. Buds were placed in one drop of propionic carmine and the anthers were removed. The anthers were gently squashed with a flat spatula. All visible plant tissue was removed from the slide, leaving the propionic carmine containing suspended pollen mother cells (PMC), meiotic material, tetrads, or pollen, depending

Table 4.1. *Desmodium heterocarpon* and *D. ovalifolium* lines and hybrids examined for tetrad formation, pollen stainability, and meiotic behavior.

Introduction Numbers [a]				
UF	IRFL	CIAT	Species[b]	Cross
Parental lines				
20	588, 6102	365	H	
28	1946	3674	O	
143	1695, 6105	3669	H	
144	1696, 6106	3670	H/h/str	
160			O	
-	6150	13183	H/h/h	
Hybrid lines				
83-3-3				160 x 6106
83-4-2				160 x 6106
83-4-3				160 x 6106
195				160 x 6102
191				28 x 6105

[a] Plant introduction numbers are numbers assigned by Dr. K. H. Quesenberry, Agronomy Department, University of Florida (UF), by Dr. A. E. Kretschmer, Jr., AREC, Ft. Pierce, Florida (IRFL), and by the Tropical Pastures Program, Columbia (CIAT).

[b] Species: H = species *heterocarpon*
O = species *ovalifolium*
H/h/h = species *heterocarpon* subspecies *hetercarpon* variety *heterocarpon*
H/h/str = species *heterocarpon* subspecies *heterocarpon* variety *strigosum*

on the age of the anthers. Material was covered with a cover slip and examined briefly under a 10X objective on a Wild M20 microscope equipped with a quartz halogen light source. Material that appeared promising was then immediately destained by first heating the slide over a flame, sealing two opposite sides of the cover slip with sticky wax, flowing 45% acetic acid under the cover slip, then sealing the remaining two sides with sticky wax. The material, once covered with a cover slip, was not squashed as this tended to disrupt cells and disorganize cellular contents. The entire staining and destaining process was limited to ten minutes as this was the time differential between which chromatin took up stain and the surrounding cytoplasm became stained.

Tetrads were counted while still enclosed within the clear envelope within which they developed. Tetrads were evaluated on whether they contained four equal sized, stained cells (normal) or they contained three or fewer normal sized, stained cells (abnormal).

Pollen was collected at 10:00 A. M. from greenhouse grown plants. Untripped flowers were tripped in a manner that caused pollen to be deposited on slides, which was then covered with a cover slip. When flowers had self-tripped before 10:00 A. M., pollen that remained on the anthers was shaken onto the slides. Pollen was stained with propionic carmine within four hours of collection, and scored immediately after staining using a 10X objective on a light

microscope. Normal sized pollen that absorbed stain was classified as stained, whereas abnormally small or shriveled pollen that did not absorb stain was classified as shriveled.

Results

Results of meiotic analysis for D. ovalifolium U.F. 160, D. heterocarpon 6106, and three F_1 hybrids are shown in Table 4.2. Both parental lines and their three hybrids showed regular first division segregation. Hybrid 83-4-3 had one cell out of twenty that had two laggards at metaphase I. All other cells showed regular pairing at metaphase I (Figure 4.1.).

Hybrid 191 (U.F. 28 x 6105) showed some abnormal pairing at metaphase I and some abnormal segregation during first anaphase division (Table 4.2.). Parental line U. F. 160 had normal metaphase I pairing and first division segregation. Parental line 6102, D. heterocarpon, had normal metaphase I pairing (Figure 4.2.), but had one cell out of twenty that had two laggards during first division segregation. Five of twenty cells of F_1 191 had two univalents at metaphase I (Figure 4.3.). The same line had four cells with one laggard, one cell with two laggards, and one cell with five laggards at first division segregation (Figure 4.3.).

Table 4.2. Chromosome configerations at metaphase I and first division segregation of parental lines and hybrid lines.

Line	Met I			First Division Segregation		
	No. of cells	No. of cells with 11 II	10 II+ 2 I	No. of cells	No. of cells with Normal	Laggards (No. of)
D. ovalifolium						
U.F. 160	20	20	0	20	20	0
U. F. 28	20	20	0	20	20	0
D. heterocarpon						
6106	20	20	0	20	20	0
6102	20	20	0	20	19	1(2)
6105	16	14	2	20	19	1(6)
Hybrids:						
(160 x 6102)						
83-3-3	20	20	0	20	20	0
83-4-2	20	20	0	20	20	0
83-4-3	20	19	1	20	20	0
(160 x 6102)						
195	20	20	0	20	18	1(1) 1(3)
(28 x 143)						
191	20	15	5	20	14	4(1) 1(2) 1(5)

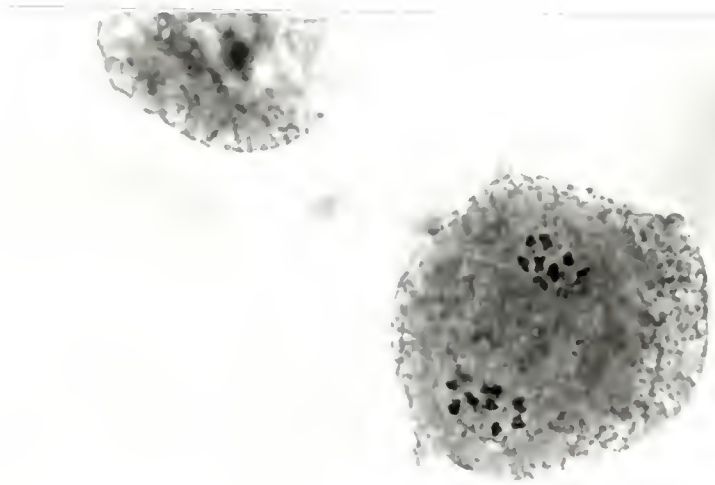
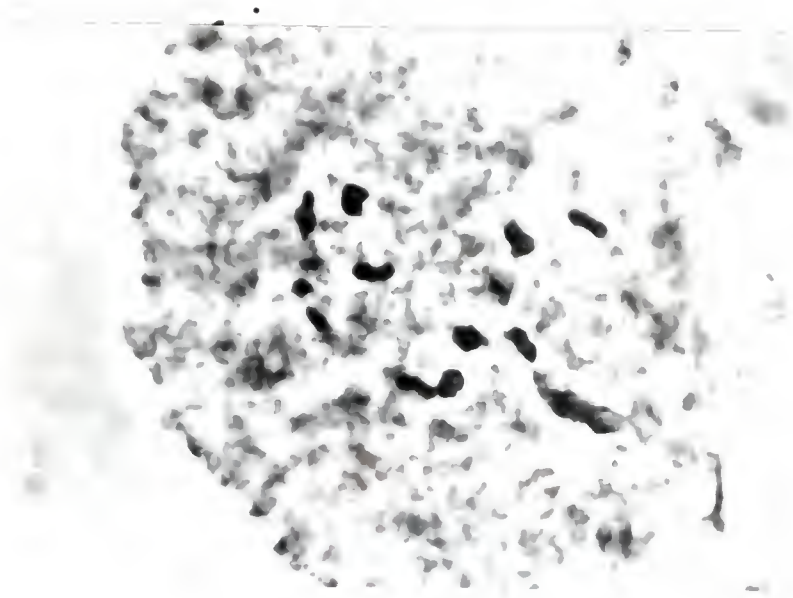


Figure 4.1. First division segregation in 6106 with no laggards.

A.



B.

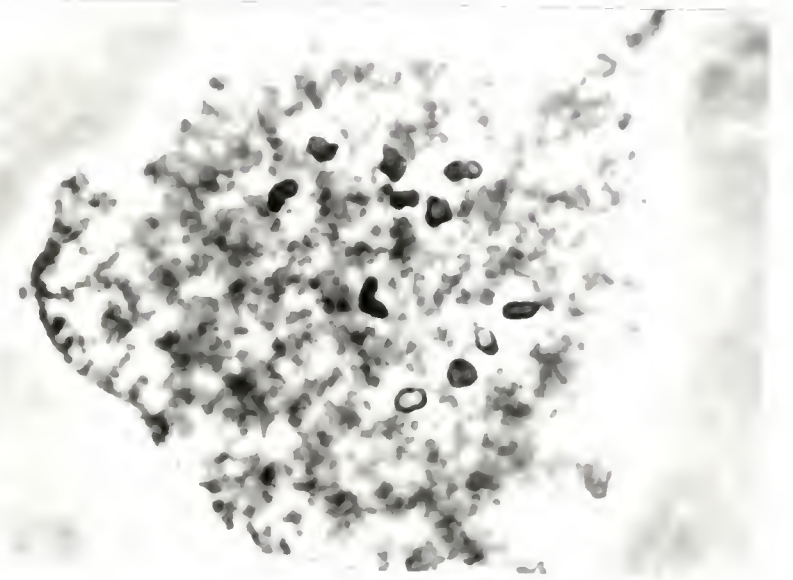
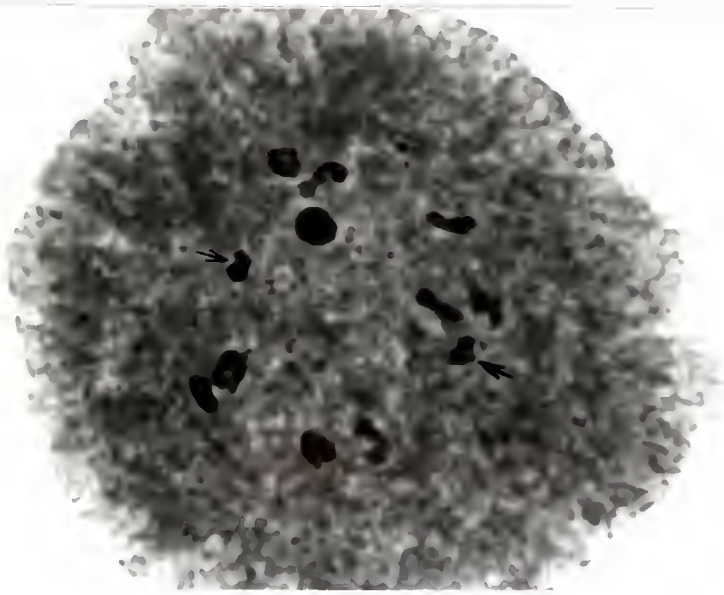


Figure 4.2. Metaphase I pairing.
A. Line 6102 showing 11 bivalents.
B. Hybrid 83-3-3 showing 11 bivalents.

A.



B.

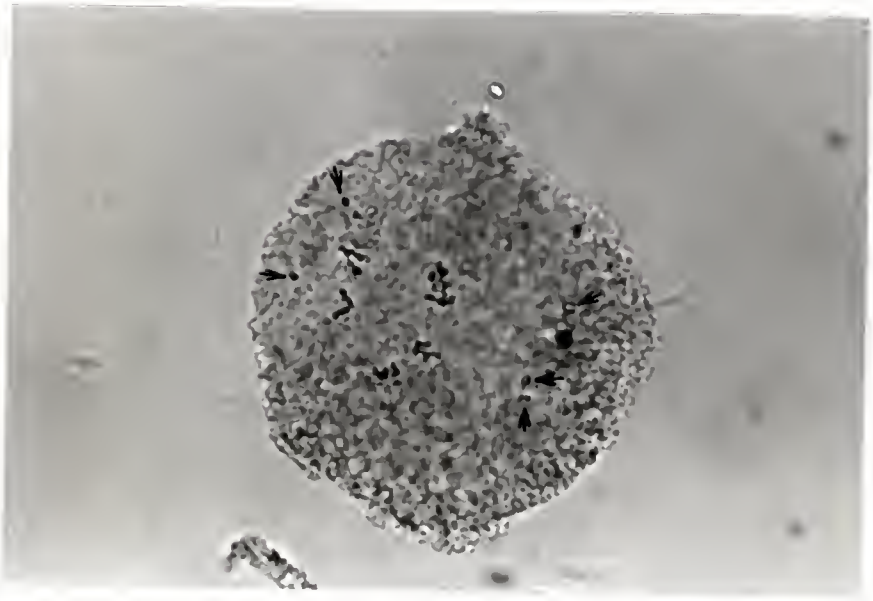


Figure 4.3. Meiosis in hybrid 191.
A. metaphase I pairing with univalents (arrows).
B. First division segregation with five laggards (arrows).

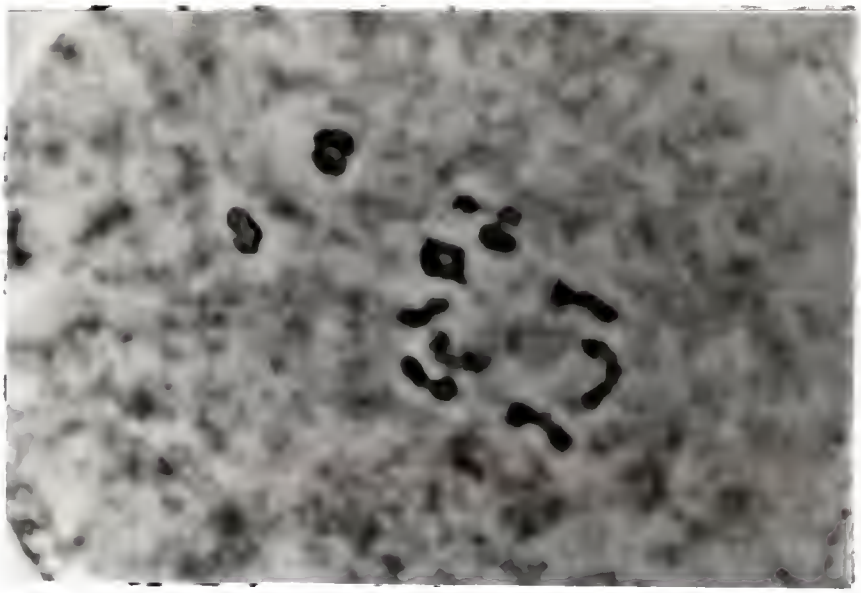
Parental line U.F. 28 (D. ovalifolium) was regular in both metaphase one pairing (Figure 4.4.) and in first division segregation. Parental line 6105 showed two cells with univalent chromosomes at metaphase 1 (Figure 4.4.) and one cell with six laggards at fist division segregation (Figure 4.4.). Hybrid 195 had normal pairing during metaphase I but had two of twenty cells that showed laggards during first division segregation (Table 4.2.). One of these cells had one laggard (Figure 4.5.) while the other had three laggards (Figure 4.5.).

Tetrad formation and pollen stainability are shown in Table 4.3. Lines 6102, U.F. 28, and hybrid 195 had 100% normal tetrads of the samples counted. Pollen stainability was high in all cases, ranging from 75% in hybrid 83-3-3 to 100% in accession 6106.

Discussion

The results for the two D. ovalifolium lines (U.F. 160 and U.F. 28) showed no problems with chromosome pairing during metaphase I or first division segregation indicating a complete complement of homologous chromosomes. Tetrad formation was normal and pollen viability was high, as would be expected. Desmodium heterocarpon lines 6102, 6105, and 6106 were only slightly less uniform in their meiotic behavior. Line 6106 was taxonomically ranked as D. heterocarpon ssp. heterocarpon var. strigosum by CIAT. The

A.



B.

Figure 4.4. Meiotic chromosome configerations.
A. Metaphase I pairing in parental line U.F. 28.
B. Diplotene pairing in parental line 6105

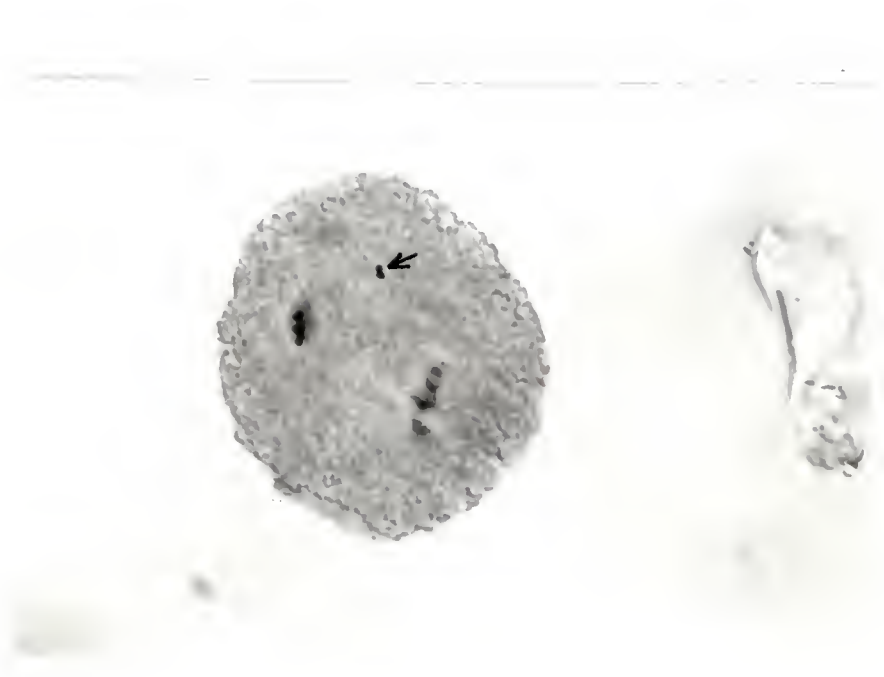


Figure 4.5. First division segregation in hybrid 195 showing one laggard (arrow).

Table 4.3. Tetrad formation and pollen stainability in greenhouse grown D. ovalifolium and D. heterocarpon lines and hybrids.

Line	Tetrads[a]				Pollen[b]			
	No. of Cells	Nor	Abn	%Nor	No. of Cells	Std	Shr	%Std
IRFL 6102	24	24	0	100	200	181	19	90
IRFL 6105	57	53	4	92	154	142	12	92
IRFL 6106	50	49	1	98	100	100	0	100
IRFL 6150	-	-	-	-	200	198	2	99
U.F. 28	60	60	0	100	100	96	4	96
U.F. 160	67	65	2	97	100	95	5	95
Hybrids:								
83-4-3	50	47	3	94	268	225	43	83
83-4-2	99	90	9	90	268	225	43	83
83-3-3	62	55	7	88	200	150	50	75
191	53	51	2	96	2828	2407	421	85
195	50	50	0	100	1892	1772	120	94

a Nor = Normal
Abn = Abnormal

b Std = stained
Shr = Shriveled

remaining two lines were classified only to the species level of D. heterocarpon. Line 6106 paralleled the D. ovalifolium lines in that it had normal pairing during meiosis and normal segregation at first division. Line 6105 had two out of sixteen cells with univalents at metaphse I and one cell out of twenty with six laggards at first division segregation. Tetrad formation and pollen stainability were both 92% for this line. Line 6102 had only one cell with laggards and no univalents at metaphase I. Its tetrad formation was as expected, 100% normal, and its pollen stainability was high (90%).

Hybrids between D. ovalifolium and D. heterocarpon have been somewhat difficult to obtain (Quesenberry and McKellar, Unpublished results). The F_2 generation of hybrids 83-3-3, 83-4-2, 83-4-3 showed morphological characteristics often asssociated with hybrid breakdown including severe stunting, chlorosis, and inability to form flowers. Hybrid breakdown often occurs in wide crosses (Stebbins, 1958). Such breakdown is usually associated with some chromosome pairing problems during meiosis. This was not the case with hybrids 83-4-3, 83-4-2, and 83-3-3. The metaphase configerations of these three hybrids showed only one cell with univalents out of a total of sixty. All cells examined were normal for first division segregation. Tetrad formation was not entirely normal but still high. Pollen stainability for these three hybrids was lower than the parental lines, ranging down to 75%. Quesenberry and Taylor

(1976) reported a similar situation in a Trifolium alpestre L. x T. heldreichianum Hausskn. cross. The F_1 hybrid had a low incidence of univalents at metaphase I and few micronuclei at the quartet stage. Nonetheless it had a mean pollen stainability of 35% less than the parent lines, and low seed set and F_2 seedling vigor.

Hybrids 191 and 195 showed more meiotic abnormalities than hybrids 83-3-3, 83-4-2, and 83-4-3. These two F_1 hybrids appear morphologically similar to the D. heterocarpon agronomic type male parent with strongly marked, pubescent leaves and upright growth habit. Hybrid 195 had two out of twenty cells with laggards. Hybrid 191 had five out of twenty cells with univalents at metaphase I and six out of twenty cells with laggards at first or second division segregation. Surprisingly, normal tetrad formation was high for both lines with hybrid 195 having 100% normal tetrads. Pollen stainability was also high for both hybrids. Pollen production, as the end result of the meiotic process, would be expected to be reduced by problems originating from lack of chromosome pairing and segregation. The high pollen stainability levels obtained for the five hybrids in this experiment would argue against extreme evolutionary distance between the two species D. ovalifolium and D. heterocarpon. Conversely, Hacker (1968) noted highly variable pollen stainability which he attributed to environmental factors. It may be desirable to test more pollen over a wider range of environmental conditions (including conditions representative

of the extremes of the greenhouse and field environment) to determine what, if any, environmental factors might be influencing pollen viabiliy. Hacker (1968), as previously mentioned, also noted that 75% pollen stainability when tetrad formation was 25% abnormal.

Initial examination of meiosis and tetrad formation was complicated by nucleosome formation that mimicked micronuclei (Figure 4.6.). Second division segregation would occur, followed by chromatids condensing as small, individual nucleosomes within one nuclear membrane. This is possibly an artifact of the preparation technique but nevertheless does not appear to influence eventual normal tetrad formation. Chromatin material within each nuclear membrane of the tetrads eventually merged into one nucleus. Different lines gave different results for this trait, so that it seems unlikely that this is an artifact of preparation.

An explanation for low success rates in crosses between D. ovalifolium and D. heterocarpon is not obvious from cytogenetic data or pollen stainability. In all cases, pollen stainability was high. Thus parental lines should have been adequate as male parents. Female parental lines, when selfed, produced seed, thus these lines were adequate as female parents. If the contributions of male and female lines are functional, yet hybrids are difficult to obtain, the problem maybe a lack of chromosome homology between D. ovalifolium and D. heterocarpon. Slight irregularities as might be caused by lack of chromosome homology, were observed

Figure 4.6. Multiple nucleosomes in 83-4-2.

in meiosis of some F_1 hybrid lines, indicating possible evolutionary distance between the two species.

The above results indicate that problems with hybridization appear related to environmental factors, and to crossing techniques and methods. There appears to be no major barrier to transfer of genetic information between the D. ovalifolium and D. heterocarpon types. Thus one should be able to transfer resistance to the desired agronomic type.

CHAPTER V
CONCLUSIONS

Caged D. ovalifolium lines set few, if any, seed. This could mean that flowers need to be tripped, particularly because tripped D. ovalifolium lines in the greenhouse set seed, or that D. ovalifolium lines need cross pollination, which is less likely. Incidental cross pollination, although not required for successful seed set, might be expected if flowers need to be tripped, presumably by insects. Differences in flowering time effectively isolated D. heterocarpon from D. ovalifolium in Gainesville. Although D. heterocarpon is capable of producing flowers at the same time as D. ovalifolium, the D. heterocarpon plants generally have stopped flowering earlier after having produced large quantities of seed.

Desmodium heterocarpon and D. ovalifolium, although separated by morphological and biochemical differences, appear to have no major crossing barriers. In spite of the opportunity for outcrossing, the D. ovalifolium lines show little variation for morphological traits, particularly for unmarked leaves. Desmodium heterocarpon types show the same uniformity, in that, when selfed, D. heterocarpon types do not segregate for marked leaves. A 9:7, marked:unmarked, F_2 hybrid ratio indicates that marked leaves are not a single gene controlled dominant trait. A test combining

isozyme marker examination of potential hybrids could confirm whether all hybrids in an unmarked leaf x marked leaf cross have marked leaves. Another possibility would be using flower color to verify the effectiveness of marked leaves as an indicator of successful hybridization, although the current D. ovalifolium germplasm does not include any white-flowered lines.

Isozyme electrophoresis for phosphohexose isomerase (PHI) distinguished between a D. ovalifolium line, a D. heterocarpon subspecies heterocarpon var. strigosum, and a D. heterocarpon subspecies not specified. The subspecies unspecified line (6102, 'Florida' carpon desmodium) was nonetheless morphologically very different than the strigosum variety and similar to the heterocarpon agronomic type in growth habit and in having clearly marked leaves. Variation in staining for PHI for five D. ovalifolium plants of line U.F. 160 indicated that this line is not homogeneous. This lack of uniformity in an D. ovalifolium line might also be expected based on its pollination biology.

Cytological studies indicated that failure of chromosome pairing during meiosis did not reduce fertility in F_1 hybrids. Apparent general chromosome homology indicated that parental lines are similar, though not identical, in chromosome compliment. Limiting factors for successful crossing in the greenhouse may be environmental conditions such as humidity or temperature. An experiment addressing

these environmental parameters using growth chambers might resolve these questions.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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December 1988

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